ROLE OF THE THALAMIC NUCLEI IN ODOUR-PLACE ASSOCIATION LEARNING

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Abstract

A common view of diencephalic amnesia is that a single diencephalic structure is responsible for the memory impairment, but an alternative view is that different diencephalic structures contribute to the memory impairment in subtly different ways. This study directly compared the effects of highly selective lesions to three thalamic aggregates (the AT, MT and LT) on an odour-place paired-associate task and a spontaneous object recognition task and used a novel quantitative analysis to calculate the damage caused by these lesions. AT and LT, but not MT lesions, severely impaired performance on the odour-place paired-associate task. Spatial probe trials introduced at the end of the odour-place paired-associate task suggested that animals may use a combination of allocentric and egocentric strategies to solve the task. No group (including controls) showed clear detection of object or object-in-place changes in the spontaneous object recognition task. The impairment in odour-place paired-associate learning in the AT group is consistent with previous research (Sziklas and Petrides, 1999) and supports Aggleton and Brown's (1999) proposal that the AT is part of an 'extended hippocampal system'. The impairment in the LT group provided new insight into the potential role of the LT in pattern association. Findings from the spatial probe tasks and the spontaneous object recognition task highlight the need for future studies to control for factors that could potentially affect performance in these tasks, such as the use of egocentric response strategies and innate object preference. The results of this study provide new information regarding the role of the thalamic nuclei in pattern association processes, and suggest that traditional models of memory function (for example, Kesner, 1998; White & McDonald, 2002) may need to be revised to take into account the important role of the thalamic nuclei in memory.

1. Introduction

1.1 General introduction

Memory impairment exists in many human disorders. In addition to cases of selective amnesia, it is a significant component in alcoholism, dementia and associated neurological disorders. These impairments can be caused by damage to a number of brain structures. Attention has focussed predominantly on the role of medial temporal lobe structures, particularly the hippocampus, in memory deficits (Gilbert & Kesner, 2002, 2003; Squire & Knowlton, 2000). While the hippocampus may be involved in several different aspects of memory, its exact functional role is unclear (Alvarez, Wendelken, & Eichenbaum, 2002). More recently, attention has increased with respect to the role of the diencephalon, especially the medial thalamus, in memory deficits in humans and other animals (Aggleton & Brown, 1999; Sziklas & Petrides, 1999). Damage to the diencephalon is generally assumed to disrupt the same declarative memory processes that are disrupted by damage to medial temporal lobe structures (Aggleton & Brown, 1999). However, the close proximity of neural structures within the medial thalamus, and the non-specific damage observed in clinical cases, means that there is uncertainty over which structures within the medial thalamus are responsible for the range of memory deficits that have been observed. Indeed, several groups of thalamic nuclei have been suggested as the critical site for diencephalic amnesia. In this case, attention has focussed predominantly on the anterior thalamic nuclei (AT), the mediodorsal nuclei (MD) and the intralaminar nuclei (IL) in the lateral thalamic region (LT).

The suggestion that the anterior thalamic nuclei (AT) are the key site for diencephalic amnesia is based on the idea that the essential features of episodic memory and diencephalic amnesia may rely on connections between the hippocampus and AT (Aggleton & Brown, 1999). Numerous animal studies have shown deficits in learning and memory after AT lesions, particularly when a spatial component is involved. For example, there is now good evidence that AT lesions are associated with memory deficits in radial arm maze studies (Moran & Dalrymple-Alford, 2003; Sziklas & Petrides, 1999). Rats with AT lesions also show impaired learning relative to controls in an allocentric object-place paired-associate task requiring associations between an object and a spatial location, but not in an egocentric object-place paired associate task requiring associations between an object and a left or right body turn (Sziklas & Petrides, 1999). While the involvement of the AT in spatial memory is presumed to be due to connections between the AT and the

hippocampus, the role of the hippocampus, and indeed the AT, in spatial and non-spatial memory requires further clarification (Aggleton & Brown, 1999; Eichenbaum, 1999).

Other evidence, however, suggests that damage to either the IL or the mediodorsal nuclei (MD) may be responsible for diencephalic amnesia. For example, Gaffan and Parker (2000) demonstrated that MD lesions in rhesus monkeys impaired both scene learning and object-reward association memory. Conversely, Burk and Mair (1998) demonstrated that lesions to the IL impaired learning on a delayed match-to-sample (DMTS) task, even after 4000 learning trials, while MD lesions had no effect on learning.

An alternative view is that no single thalamic region is responsible for diencephalic amnesia. Instead, different thalamic regions may contribute to diencephalic amnesia in different ways. For example, Aggleton and Brown (1999) suggested that anterograde amnesia may be caused by damage to two memory systems, one underlying recall and one underlying familiarity-based recognition. The recall system was presumed to rely on connections between the hippocampus and the AT, while the recognition system relies on connections between the perirhinal cortex and the MD. According to this model, damage to any area within each system will result in similar impairments. A common source of support for this model is the finding that lesions to the AT and hippocampus (both included in the recall system) cause similar impairments in spatial memory (Aggleton, Neave, Nagle, & Hunt, 1995; Moran & Dalrymple-Alford, 2003).

Recent work at the University of Canterbury has also supported the notion that different thalamic areas contribute to memory in subtly different ways. The previous study by Mitchell and Dalrymple-Alford (2005) is the only one thus far to directly compare the effects of selective lesions to one of three aggregates of thalamic nuclei on learning and memory. They compared the effects of lesions to an anterior thalamic aggregate (AT), a posterior medial thalamic aggregate (MT; midline thalamic region) and a lateral thalamic aggregate (LT; IL plus lateral MD) on a series of memory tasks. AT lesions, but not MT lesions, impaired working and reference memory performance on a radial arm maze task. LT lesions impaired working memory performance, but only slightly. MT lesions, but not AT or LT lesions, impaired performance on a reward magnitude task. Finally, there was also some evidence that both MT and LT lesions, but not AT lesions, impaired memory for the temporal order of familiar objects. The main aim of the current study was to extend this recent work by examining the effects of AT, MT and LT lesions on an odour-place paired associate task in which rats learn arbitrary associations between an odour and a spatial location. Paired-associate learning is commonly regarded as an important model of episodic-like memory in humans and animals,

particularly when it involves a spatial component (Aggleton & Pearce, 2001). No previous study has directly compared the effects of different thalamic lesions on a paired-associate learning task. Given the task employed, some insight into the role of the AT, MT and LT thalamic regions in spatial and odour memory *per se*, in addition to associative memory, was also expected.

The effects of AT, MT and LT thalamic lesions on a modified version of the spontaneous object recognition were also examined. An advantage of the spontaneous object recognition paradigm used in the present study is that it allowed analysis of both spatial and non-spatial changes in objects and their locations, providing information on the role of the thalamic nuclei in both spatial and non-spatial object memory.

1.2 The neuroanatomical and neuropsychological basis of diencephalic amnesia

1.2.1 Human studies

Anterograde amnesia is characterized by an inability to form new episodic memories, while intelligence and many other cognitive functions are relatively unaffected (Squire & Knowlton, 2000). Diencephalic damage associated with amnesia can result from infarction, tumor, vascular accident or the alcoholic Korsakoff syndrome. Human cases of diencephalic amnesia have been reported to have damage to regions that include the AT, MD and mammillary bodies (Harding, Halliday, Caine, & Kril, 2000; Kapur, Thompson, Cook, Lang, & Brice, 1996; Knight & Longmore, 1994; Mair, Warrington, & Weiskrantz, 1979; Mayes, Meudell, Mann, & Pickering, 1988; Victor, Adams, & Collins, 1971; von Cramon, Hebel, & Schuri, 1985). Both cell bodies and fibre pathways in the thalamus that connect various cortical and sub-cortical structures show damage in these cases.

An excellent early example reported evidence from a large group of patients with Korsakoff syndrome. Of the 43 patients whose thalamus was available for post-mortem analysis, 38 had damage to the MD. The 5 patients without MD damage were the only ones who apparently showed no clinically severe memory impairment (Victor et al., 1971). However, these 5 patients all had damage to the mammillary bodies, leading to the conclusion that damage to the MD (either alone or in combination with mammillary body damage), but not the mammillary bodies alone, is the basis of diencephalic amnesia. However, the authors noted that some adjacent areas of these patients' brains (including the pulvinar, geniculate bodies and habenular nuclei) were unavailable for analysis, and therefore their involvement in memory impairment could not be ruled out. Subsequent studies have,

however, indicated that damage to the mammillary bodies and paratenial nucleus, without MD damage, may in fact be sufficient to cause amnesia (Mair et al., 1979; Mayes et al., 1988). A more recent example is that of Harding, Halliday, Caine and Kril (2000). These authors studied cell loss in the MD, AT and mammillary bodies in four groups of patients: non-alcoholic controls; alcoholic controls; alcoholics with Wernicke's encephalopathy but no amnesia; and alcoholics with the amnesic Korsakoff syndrome. Compared to controls, alcoholics with Wernicke's encephalopathy or Korsakoff syndrome had substantial degeneration of the MD and mammillary bodies. However, only the patients with Korsakoff syndrome had damage to the AT. The authors concluded that while Korsakoff syndrome involves lesions to the MD, AT and mammillary bodies, it is the AT lesions that may be primarily responsible for the amnesia.

Despite such promising results, research attention has turned away from the study of human cases given their inherent limitations. In cases of human diencephalic damage, there is often a long period between assessment of memory impairment and post-mortem analysis of brain damage, and the size and structure of lesions may change over this time period (Mayes et al., 1988). Furthermore, for most patients there is no record of pre-morbid memory function. The small size and close proximity of diencephalic structures create further problems. Diencephalic damage is rarely localised, especially in humans, and may affect fibre tracts and pathways connecting distant brain areas. These limitations have led to the development of animal models of diencephalic amnesia that attempt to eliminate some of these problems. By using animal models, the size and location of damage can be experimentally determined, pre-morbid assessment is freely available, and the length of time between damage and brain analysis can be controlled.

1.2.2 Animal studies: The possible role of the thalamus in multiple memory systems

Animal models of diencephalic amnesia attempt to make selective damage to various structures within the diencephalon or mimic the alcoholic Korsakoff syndrome and measure the effects on learning and memory tasks. Damage can be produced in a number of ways, with lesions produced by excitotoxins, electrolytic or radiofrequency current, excision or ablation, or by the pyrithiamine-induced thiamine deficiency (PTD) model.

However, the results of these animal studies continue to fail to confirm the traditional view that there is a single key site within the diencephalon that is responsible for diencephalic amnesia. As mentioned earlier, some evidence has suggested that the AT may be the key site (Moran &

Dalrymple-Alford, 2003; Sziklas & Petrides, 1999), while other evidence has suggested that the MD (Gaffan & Parker, 2000; Victor et al., 1971) or IL (Burk & Mair, 1998) may be critical.

As suggested earlier, both the human and animal evidence indicates that there is no single key site responsible for diencephalic amnesia. Instead, different thalamic areas contribute to memory in subtly different ways. Hence various alternatives propose that intact performance on a memory task may rely on a number of different thalamic areas or systems, each underlying a different type of memory or memory-related factors such as arousal or executive functioning (Aggleton & Brown, 1999; Mitchell & Dalrymple-Alford, 2005; Van der Werf, Jolles, Witter, & Uylings, 2003). In cases of diencephalic amnesia, the amount of damage to various structures within the thalamus would then determine the specific characteristics or range of memory impairment.

It is now generally accepted that the brain employs multiple neural systems to process different kinds of memory. It is not feasible here to discuss all these options in relation to diencephalic amnesia, so three main examples will suffice. Aggleton and Brown's influential view was previously mentioned, which suggests a hippocampal-based recall system and a PRC-based recognition system (Aggleton & Brown, 1999). A more classic example is that of White and McDonald (2002). Their model involves three independent parallel memory systems, each based around a central structure: the hippocampus, amygdala and dorsal striatum systems. Although the three systems have access to the same information, each system represents a different type of association between the elements (stimulus events, responses and rewards) of a memory task. The hippocampus system represents stimulus-stimulus associations, the amygdala system represents stimulus-reward associations, and the dorsal striatum system represents stimulus-response associations. White and Macdonald's model (2002) is superficially similar in many respects to a third example. Kesner's model (1998) emphasises the influence of different memory systems with respect to specific memory attributes, such as space, time, response, sensory perception, affect, and, in humans, language. According to Kesner's model, there are three main memory systems (event-based, knowledge-based and rule-based) but these are not to be confused with White and McDonald's three systems; the overlap is more to do with the idea that different attributes are likely processed by the hippocampus, amygdala and caudate. In each of Kesner's memory systems, different sets of neural structures undertake various types of processing related to each attribute. Within event-based memory, the hippocampus processes information related to language, time and space, the caudate processes information related to response, the amygdala processes information related to affect, and the PRC processes information related to sensory-perception. The model also

specifies a number of processes common to the three systems. Most relevant is the idea that event memory for each type of attribute requires a set of processes, which are labeled pattern association, pattern separation, pattern completion (consolidation) and working memory. For example, Kesner has provided evidence that the hippocampus is involved with pattern association processes, but only when the task includes space, time or (in humans) language attributes (Gilbert & Kesner, 2002; Kesner, 1998). This latter evidence is especially relevant to the current study. The presumed similarity of hippocampal and AT lesions suggests a possible role for the AT in pattern association processes also, which is tested by paired-associate tasks. Thus far, the comparative evidence on AT, LT and MT lesions (Mitchell and Dalrymple-Alford, 2004, 2005), which showed that different thalamic regions contribute to memory in different ways, can be construed as supporting a role for thalamic regions in the context of either White and McDonald (2002) or Kesner's (1998) multiple memory system models.

1.2.3 Structure and connections of the thalamic nuclei

The concept that different thalamic regions may play a role in different memory systems finds support from work on the neural connections associated with the nuclei in the limbic thalamus and adjacent areas. Much of the problem of interpretation of related lesion studies is, then, the specificity of the lesions employed. The location, size and specificity of lesion vary considerably between studies (see section 1.7 for more discussion of this issue). As mentioned, the thalamic regions targeted in memory studies include the AT, MD, IL and the lateral internal medullary lamina (L-IML). As stated earlier, the present study produced highly specific lesions to three aggregates of these thalamic nuclei: an anterior thalamic aggregate (AT); a posterior medial thalamic aggregate (MT), which includes the medial and central MD; and a lateral thalamic aggregate (LT), which includes the IL and lateral MD. Each aggregate has a number of principal cortical and sub-cortical connections, so these are reviewed briefly here. Figures 1.1, 1.2 and 1.3 (pp8-9) give conceptual diagrams of these differing connections between the AT, MT and LT and cortical and sub-cortical areas. Their connections are described in more detail below.

The AT comprises the anteroventral, anteromedial and anterodorsal thalamic nuclei. The AT's most prominent neural connections are the dense reciprocal pathways between the AT and the hippocampus via the retrosplenial cortex and the subicular region (Shibata, 1998), although the AT also projects to other areas, including the temporal and prefrontal cortex (Aggleton & Brown, 1999).

This neuroanatomy stimulated research that led to the view that the AT are part of an 'extended hippocampal system' critical for recall that also includes the fornix and mammillary bodies (Aggleton & Brown, 1999).

The MT comprises the central and medial segments of the MD and the intermediodorsal nucleus, and projects reciprocally to the PRC and prefrontal cortex (Aggleton & Brown, 1999). The MT also has connections with the entorhinal cortex and the amygdala (Groenewegen, 1988). This neuroanatomy has led to the view that the MT may play a role in an amygdala-based memory system (Gaffan & Murray, 1990; Mitchell & Dalrymple-Alford, 2005).

The LT comprises the IL as well as the lateral segments of the MD. This aggregation has generally been ignored in favour of the more traditional division between the IL and MD. However, the IL and lateral MD are grouped together in the LT aggregate on the basis of their respective projections to the caudate putamen and dorsomedial striatum (Cheatwood, Reep, & Corwin, 2003). The IL have also traditionally been regarded as 'non-specific' nuclei that do not project to specific areas, but rather have a widespread distribution of their efferent fibres. However, the recent development of sophisticated tracing techniques has shown that the IL do have specific projections to areas of the striatum and areas of the cortex, including prefrontal association and posterior parietal areas (Groenewegen & Berendse, 1994). Thus part of the role of the LT may be in terms of a dorsal striatal response memory system (Mitchell & Dalrymple-Alford, 2004). Another body of evidence, however, suggests that the IL may be part of a system, this time including the ventral striatum, that is involved in some types of spatial memory (Porter, Koch, & Mair, 2001). Furthermore, lesions to the fibre system that comprises the L-IML, within the LT region, may cause damage in other brain regions that is not a direct result of the lesion procedure, including the anteroventral AT and mammillary bodies, hence potentially disrupting a wide range of cognitive functions (Savage, Sweet, Castillo, & Langlais, 1997). Animal studies using the PTD model have identified that thiamine deficiency (similar to that seen in the alcoholic Korsakoff syndrome) can cause damage to areas in the LT region, including the L-IML, as well as the AT region (Mumby, Cameli, & Glenn, 1999). It appears once again, then, that perhaps poor specificity of lesion to the LT or to targets within the LT region have contributed to uncertainty and mixed views on the role of the LT and its components.

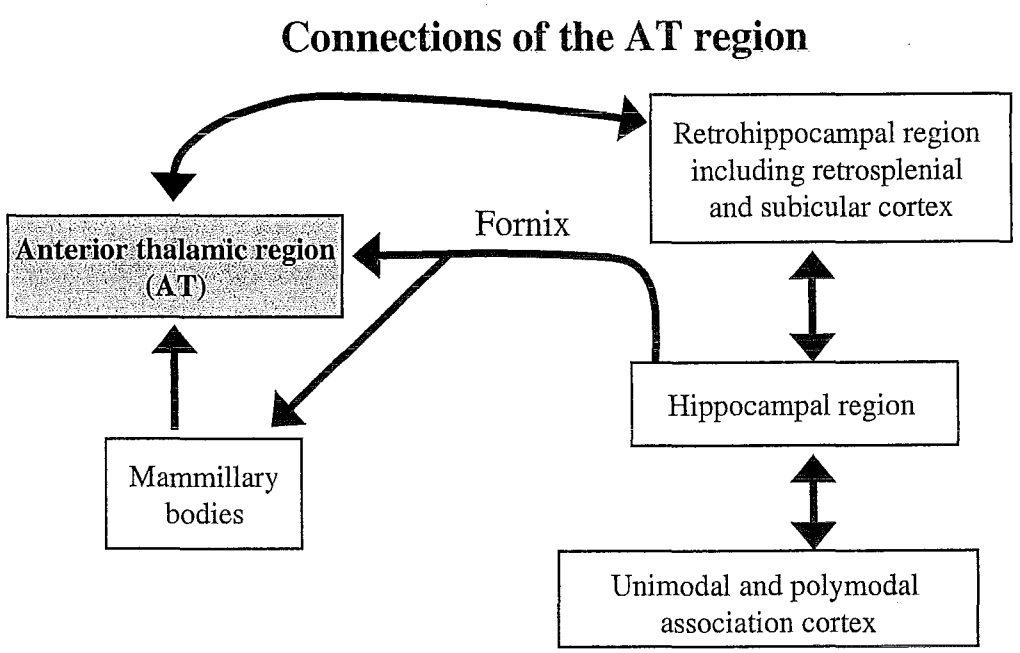


Figure 1.1. Conceptual diagram showing the connections of the anterior thalamic (AT) region. Reproduced with permission from Dalrymple-Alford (2005) and adapted from Aggleton and Brown (1999).

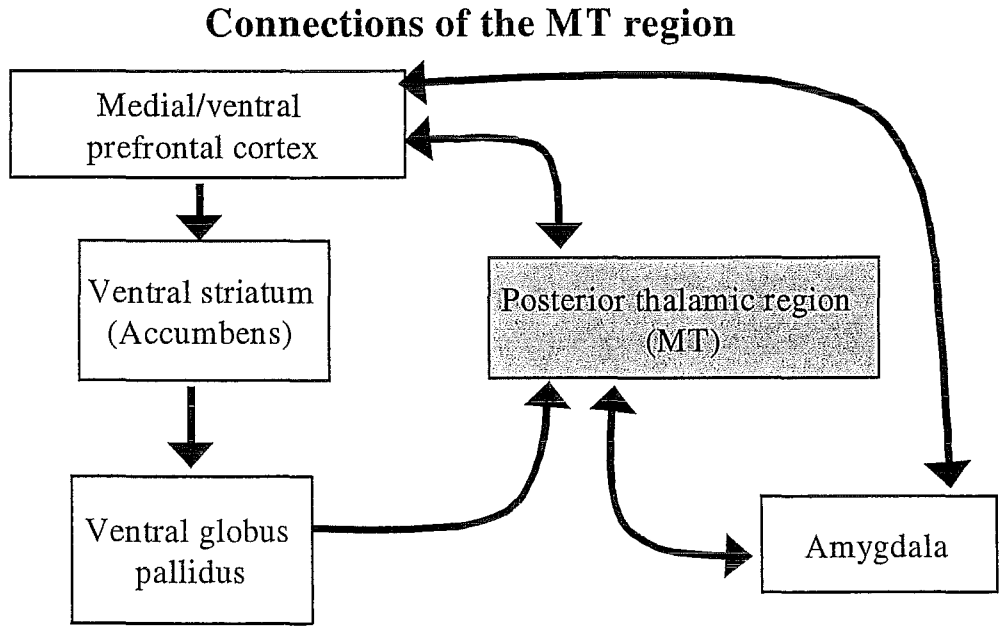


Figure 1.2. Conceptual diagram showing the connections of the posteromedial (MT) thalamic region. Reproduced with permission from Dalrymple-Alford (2005) and adapted from Aggleton and Brown (1999).

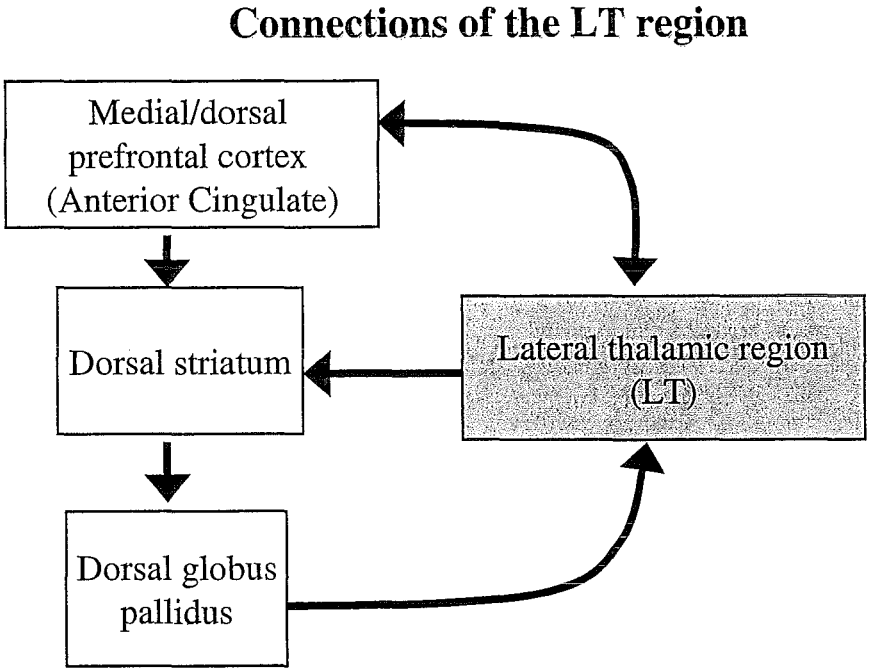


Figure 1.3. Conceptual diagram showing the connections of the lateral thalamic (LT) region. Reproduced with permission from Dalrymple-Alford (2005) and adapted from Aggleton and Brown (1999).

1.3 Memory attributes presumed to be involved in the current study

Any given memory task may involve a number of memory attributes, relevant cues, strategies and memory systems, and it is presumed that memory impairments seen after brain damage will vary according to the specific factors involved in the task. The main task in the current study examined the effects AT, MT and LT lesions on performance in a go/no-go odour-place paired-associate learning task. The task required rats to learn arbitrary associations between spatial locations and odours. Hence, the task involved "pattern association" processes. Tasks that involve pattern association processes are of particular value because they are presumed to reflect episodic-like memory in non-human animals. The task also involved both 'spatial' and 'odour' attributes. After acquisition of the odour-place paired-associate task, a series of probe trials was added in which the start position was switched to the opposite side of the apparatus. The aim of introducing the probe trials was to examine the use of allocentric and egocentric spatial strategies in solving the odour-place paired associate task. Subsequently, rats were tested on a go/no-go simple discrimination task for either the two odours or the two spatial locations used in the odour-place

paired-associate task. The aim of the simple discrimination tasks was to determine whether impaired performance on the odour-place paired associate task was due to an inability to discriminate between the odours or places used, or an inability to inhibit responses. Although these latter tasks involved an odour or spatial attribute, they did not involve any paired-associate learning and therefore serve as a comparison for the paired-associate task. The third task employed a sequence of spontaneous object recognition tests that examined memory for familiar objects and their spatial locations. As this series of tasks, overall, can be assumed to involve spatial, odour and object memory, the role of the thalamic nuclei in memory for spatial locations, odours and objects will be discussed in the following sections.

1.4 The role of the thalamic nuclei in spatial memory

Table 1.1 (pages 14-22) provides a summary of studies of the effects of thalamic lesions in tasks involving a spatial attribute. For ease of comparison, all tasks in each study (including non-spatial tasks) are listed. While the majority of these studies have focused on the AT, the roles of other thalamic regions have also been examined, including the MD, L-IML, and IL.

1.4.1 The AT region

The most common test of spatial memory after AT lesions has been the radial arm maze (RAM) (see, for example, Aggleton, Hunt, Nagle, & Neave, 1996; Alexinsky, 2001; Byatt & Dalrymple-Alford, 1996; Mair, Burk, & Porter, 2003; Mitchell & Dalrymple-Alford, 2004, 2005; Moran & Dalrymple-Alford, 2003; Sziklas & Petrides, 1999). There is even evidence that small lesions to sub-regions of the AT (the anteroventral and anteromedial nuclei) cause deficits in RAM tasks (Byatt & Dalrymple-Alford, 1996), although another study found deficits after combined anteroventral and anterodorsal AT lesions, but little or no change after small anteromedial AT lesions (Aggleton, Hunt, Nagle, & Neave, 1996). Of the studies listed in Table 1.1, the only study to find no impairment in RAM performance after AT lesions is that of Berracochea, Jarrard and Jaffard (1989). One factor that may help to explain the results of Beracochea et al is the use of egocentric strategies to solve their RAM task, which is a feasible explanation for their study because their radial arm maze lacked doors (allowing rats for example to simply always choose an adjacent arm or every third arm). In support of this explanation, recent studies have reported that AT lesions do not

produce a deficit on egocentric spatial tasks when optimal performance can be reached by relying on body-turn or local environmental cues (Aggleton et al., 1996; Sziklas & Petrides, 1999; Warburton, Baird, & Aggleton, 1997).

In addition to impairments on RAM tasks, AT lesions have been shown to impair performance on several other spatial tasks, including: forced spatial alternation (Gaffan, Bannerman, Warburton, & Aggleton, 2001; Warburton & Aggleton, 1999; Ward-Robinson et al., 2002); spatial DNMS (Aggleton & Saghal, 1993); object-place paired associate learning (Sziklas & Petrides, 1999) and Morris water maze tasks (Sutherland & Rodriguez, 1989; Warburton & Aggleton, 1999; Warburton, Morgan, Baird, Muir, & Aggleton, 1999). The finding that AT lesions impair performance on a wide range of spatial tasks suggests that the AT are involved in allocentric spatial memory, possibly due to connections between the AT and hippocampus (Aggleton & Brown, 1999).

1.4.2 The MT region

With the exception of Mitchell and Dalrymple-Alford (2004, 2005), all of the studies listed in Table 1.1 that involve lesions to the MT region have focused these lesions on the MD. However, like Mitchell and Dalrymple-Alford (2005), the current study used a non-traditional posterior medial thalamic aggregate (MT) that includes the central and medial segments of the MD and the inter-mediodorsal nucleus, but attempted not to include the lateral MD (which is included in the LT aggregate instead, see section 1.2.3).

Evidence on the role of the MD in spatial memory has been mixed. Although an early study reported that MD lesions had no effect on spatial memory (Kolb, Pittman, Sutherland, & Whishaw, 1982), Stokes and Best (1988; 1990a; 1990b) reported that MD lesions produced a severe impairment on radial arm maze tasks.

Recent work, however, failed to replicate these latter results. For example, Mitchell and Dalrymple-Alford (2005) reported that rats with MT lesions showed no impairment in working or reference memory in the radial maze. Burk and Mair (1998) found that MD lesions had no effect on place DMS. Hunt and Aggleton (1998) demonstrated that lesions confined solely to the MD had no effect on performance in the standard radial maze task and that a subgroup of MD rats that had lesions that encroached onto the adjacent anterior thalamic nuclei showed a deficit in the task. Hunt and Aggleton suggested that overlap of lesions into the adjacent AT could account for the conflicting results after MD lesions, consistent with evidence that even small lesions of the AT can

impair spatial memory (Byatt & Dalrymple-Alford, 1996). Hunt and Aggleton (1998) also reported that rats with lesions restricted to the MD were, however, impaired when the radial maze was rotated (with the baited arms remaining in the same locations), indicating that they were relying more on intramaze cues than extramaze cues. Therefore, it is likely that both the overlap of MD lesions with the AT and the specific requirements of the spatial task may account for the variation in results regarding the role of the MD in spatial memory.

1.4.3 The LT region

Several studies listed in Table 1.1 have examined spatial memory after lesions to the LT region. These studies focused on several different areas within the LT region, including the L-IML (Burk & Mair, 1998; Mumby et al., 1999; Savage, Castillo, & Langlais, 1998), IL (Mair, Burk, & Porter, 1998), IL and midline nuclei (Savage et al., 1998) and LT (IL and lateral MD) (Mitchell & Dalrymple-Alford, 2005). Nearly all of these studies have found spatial memory deficits; in some cases the deficits were severe. For example, Burk & Mair (1998) reported that IL lesions produced a deficit in place DMS that persisted throughout 8 months of post-operative training and more than 4000 DMS trials. When confined to the L-IML the lesions produced a smaller and more transient impairment. Deficits in RAM performance have also been reported after IL (Mair et al., 1998) and L-IML (Harrison & Mair, 1996; Young, Stevens, Converse, & Mair, 1996) lesions. Performance on the Morris water maze task has also been shown to be impaired after IL (Mair et al., 1998; Savage et al., 1998; Savage et al., 1997), L-IML (Savage et al., 1998; Savage et al., 1997) and combined IL and midline (Savage et al., 1998) lesions.

Only two studies listed in Table 1.1 have failed to find clear spatial deficits after lesions to the LT region. Mumby, Cameli and Glenn (1999) reported that L-IML lesions had no effect on spatial DMS in a Morris water maze. Mitchell and Dalrymple-Alford (2005) revealed that carefully localised lesions to the LT (IL and lateral MD) produced only minor effects on working but not reference memory in the radial maze, and that this impairment may have been due to associated AT damage.

There is evidence that spatial memory impairments after LT lesions may be caused by damage to connections between the IL and the striatum. Porter, Koch and Mair (2001) reported greater deficits in place DMS when a unilateral lesion to the olfactory tubercle area of the ventral striatum was accompanied by a contralateral infusion of lidocaine to the IL than when the infusion

was on the ipsilateral side. The deficits after contralateral infusion of lidocaine were of a similar magnitude to those observed after bilateral IL lesions.

Table 1.1 Studies using thalamic lesions and behavioural tasks involving a spatial memory component

Year	Authors	Lesion (species/areas-method)	Extra damage	Tasks	Training	Deficits (none, slight, moderate, severe)
2005	Mitchell & Dalrymple-Alford	Rats/AT, MT, LT-NMDA	AT- MD, IL MT- AM, AV, AD, IL LT- AM, AV, AD	RAM- working and reference	Pre-op	AT- severe, both working and reference LT- slight, working
				Reward magnitude go-no go	Post-op	MT- severe
				Temporal order for objects	Post-op	MT, LT- moderate
				SOR	Post-op	None
2004	Ridley, Baker, Mills, Green & Cummings	Monkey/ successive surgeries: surg 1= unilateral AT- NMDA surg 2= unilateral IT- excision (order of surgeries counterbalanced)	AT- MD, ventroanterior thalamic nucleus, MM reduced IT- PRC	Simple object discrimination	Pre-op	None
				Successive object discrim	Post-op	None
				Spatial discrimination	Post-op	Moderate
				Visuospatial task	Pre-op	AT, IT- None AT+IT- moderate
				Spatiovisual task	Post-op	AT+IT- Severe
				Visuovisual task	Post-op	AT+IT- Severe
				Background spatial task	Post-op	None
2003	Mair, Burk & Porter	Rats/AT- NMDA, PH- RF	AT-LD, IL, MD, CL, needle tract in H PH- EC, electrode tract in temporal, parietal, occipital cortex	RAM- spatial DNMS	Pre-op	AT- moderate, delay dep PH- slight

Table 1.1 continued

Year	Authors	Lesion (species/areas-method)	Extra damage	Tasks	Training	Deficits (none, slight, moderate, severe)
2003	Moran & Dalrymple-Alford	Rats/ AT, PRC- NMDA	AT- LD, PC, CL, CM, PT, IAM, Rh, MD, VL, reticular PRC- CA1, temporal cortex area 3, ER, postrhinal	RAM SOR Elemental cue learning Configural cue learning	Post-op Post-op Post-op Post-op	AT- severe PRC- none None PRC- slight AT- none PRC- moderate AT- none
2002	Van Groen, Kadish & Wyss	Rats/LD, LD+AD+AV- IBO	None reported	Morris water maze	Post-op	LD moderate LD+AD+AV severe
2002	Ward-Robinson, Wilton, Muir, Honey, Vann & Aggleton	Rats/AT- NMDA	Re, mid nuclei, MD, LD, DG	T-maze spatial forced alternation Sensory preconditioning	Post-op Post-op	AT- severe None
2001	Alexinsky	Rats/AT, MD- IBO RSC, PPC- excision	No details given	3/8 baited RAM reference and working memory New route- pre exposure vs none Contextual light change	Pre-op Pre-op or post-op Post-op	RSC none PPC moderate AT severe MD moderate No pre-exp: PPC, MD, AT severe Pre-exp: PPC, AT moderate AT most disrupted
2001	Gaffan, Bannerman, Warburton & Aggleton	Rats/ AT, MM, EC- NMDA, Fx- manual cut	AT- MD EC- pre- and parasubiculum, subiculum, vH, PRC, DG Fx- stria terminalis, AD, AV	T-maze spatial forced alternation Locomotor activity Y-maze constant negative pretraining Constant negative object scene discrimination	Post-op Post-op Post-op Post-op	MM- moderate AT- severe EC- slight increase Fx- large increase Fx- moderate Fx, AT, MM- better than shams

Table 1.1 continued

Year	Authors	Lesion (species/areas-method)	Extra damage	Tasks	Training	Deficits (none, slight, moderate, severe)
2001	Porter, Koch & Mair	Expt 1: Rats/ IL- bilateral lidocaine infusion	No details given	Spatial DMS	Pre-op	IL- moderate, delay dependent
		Expt 2: Rats/ OfT (unilateral)-NMDA, IL- ipsi/contralateral lidocaine infusion	No details given	Spatial DMS	Pre-op	OfT+contra- severe OfT+ipsi- slight
2001	Wilton, Baird, Muir, Honey & Aggleton	Rats/AD+LD-NMDA	AV, AM, DG	T-maze spatial forced alternation	Post-op	AD+LD- severe
				Water maze beacon	Post-op	AD+LD- severe
				Object-in-place recognition	Post-op	AD+LD- severe
				SOR	Post-op	None
1999	Mumby, Cameli, Glenn	Rats/L-IML - ELEC	AM, MD, CM, CL, VL, PC	Spatial DMS	Post-op	None
				Object discrimination	Post-op	None
1999	Sziklas & Petrides	Rat/AT- ELEC	Expt 1: IAM, LD, MD, PC, PT	Expt 1: Object-place association	Post-op	AT- severe
			Expt 2: IAM, PT, PC, PV, MD	Expt 2: Object-response association	Post-op	AT- none
			Expt 3: Used rats from expt 1	Expt 3: RAM- working memory	Post-op	AT- severe
1999	Warburton & Aggleton	Rats/AT, AT+MD-NMDA, Fx- RF	AT- Re, LD, nmd, DG, MM	SOR	Post-op	None
			AT+MD- LD, Re, nmd, MM	Morris water maze	Post-op	Fx- moderate AT, AT+MD- severe
			Fx- AV, AD, S, CC	T-maze spatial forced alternation	Post-op	Fx, AT, AT+MD- moderate

Table 1.1 continued

Year	Authors	Lesion (species/areas-method)	Extra damage	Tasks	Training	Deficits (none, slight, moderate, severe)
1999	Warburton, Morgan, Baird, Muir & Aggleton	Rats/ AT- NMDA, FF-RF	AT- MD, mid, rostral reticular, rostral IL, PT, DG, Re, LD, CM, CL, PC FF- AV, AD, septum, CC	Morris Water maze T-maze spatial forced alternation	Pre-op Pre-op	AT, FF- moderate AT, FF- moderate
1998	Burk & Mair	Rats/MD, IL, L-IML-NMDA	MD- L-IML IL- CM, MD, LD, lateral posterior and VM L-IML- PC, CL, MD, VM, LD	Spatial DMS Spatial SRL	Pre-op Post-op 8 months post-op Post-op	MD none IL severe L-IML moderate MD none IL moderate L-IML moderate IL slight
1998	Hunt & Aggleton	Rats/MD- NMDA	LD, AD, AV, mid nuclei, PV, PT, DG, needle tract in H	RAM- working memory RAM- reference memory T-maze spatial forced alternation SOR	Post-op Post-op Post-op Post-op	MD- slight-moderate MD- moderate when maze rotated None None

Table 1.1 continued

Year	Authors	Lesion (species/areas-method)	Extra damage	Tasks	Training	Deficits (none, slight, moderate, severe)
1998	Mair, Burk & Porter	Expt1: Rat/MW, H-RF	Expt1: None reported	Expt 1: Spatial DMS SRL	 Pre-op Post-op	 MW- moderate None
		Expt2: Rat/H-RF	Expt2: None reported	Expt 2: Olfactory continuous DNMS	 Pre-op	 None
		Expt3: Rat/IL-NMDA, H-RF	Expt3: IL- MD, LD, lateral posterior, VM	Expt3: RAM- standard task RAM- four forced choice	 Pre-op Post-op	 H, IL- moderate IL- moderate, delay independent H, IL- moderate, delay dependent
				RAM- DNMS	Post-op	H- moderate, delay dependent IL- moderate, delay dependent; severe when external cues minimised
		Expt4: Used rats from expts 1-3	Expt 4: Used rats from expts 1-3	Morris water maze	Post-op	H-moderate MW- slight to moderate IL- severe
1998	Savage, Castillo, Langlais	Rats/L-IML- RF, IL+ (intralaminar + midline)- IBO	L-IML- AD, AM, mid nuclei, MD, Rh, gelatinosus nuclei IL+- AT, MD, Rh, gelatinosus nuclei, Po, VL, MM	Successive object discrimination Morris water maze Morris Probe task Repeated acquisition Acoustic startle	Pre-op Post-op Post-op Post-op Post-op Post-op	None None IL+- moderate IL+- moderate IL+- moderate None

Table 1.1 continued

Year	Authors	Lesion (species/areas-method)	Extra damage	Tasks	Training	Deficits (none, slight, moderate, severe)
1997	Parker & Gaffan	Monkeys/AT, CgC-ablation	CgC- frontal pole, dorsolateral, supplementary motor cortex AT- mid, caudate nucleus, ventral anterior nucleus, Fx, MM, LD	Object-in-place	Pre-op	AT- moderate
1997	Savage, Sweet, Castillo & Langlais	Rats/L-IML, Po+PF-RF	L-IML- AM, AV, CM, Rh, gelatinosus, ventral MT, MM reduced Po+PF- MM reduced	Spatial DNMS Morris water maze Acoustic startle Passive avoidance	Pre-op Post-op Post-op Post-op	IML- severe Po+PF- long delays only IML- moderate L-IML- startle reduced None
1997	Warburton, Baird, Aggleton	Rats/AT, AT+LD-NMDA Fx-RF	AT+LD- MD, Re, reticular nucleus, DG AT- LD, MD, Re Fx- AD, AV, LD	T maze spatial forced alternation T maze allocentric alternation Egocentric discrimination	Post-op Post-op Post-op	AT, AT+LD, Fx-severe Fx- moderate AT, AT+LD- severe All groups decreased similarly when start position moved None
1996	Aggleton, Hunt, Nagle & Neave	Rats/ AM, AV+AD, AT(AM+AV+AD)-NMDA	AT- rostral mid MD, LD, MM shrinkage AV+AD- AM AM- PT	T maze spatial forced alternation T maze allocentric alternation Egocentric discrimination RAM	Post-op Post-op Post-op Post-op	AT- severe AM, AV+AD- moderate AT- severe None AV+AD- severe (correct), moderate (arm visits) AT- severe (correct), moderate (arm visits)

Table 1.1 continued

Year	Authors	Lesion (species/areas-method)	Extra damage	Tasks	Training	Deficits (none, slight, moderate, severe)
1996	Byatt & Dalrymple-Alford	Rats/ AM, AV- RF	AM- SM, PV, PT, CM, Rh, AD, AV AV- SM, Re, VL, AD, AM	RAM	Post-op	AM, AV- severe
1996	Harrison & Mair	Expt 1: Rats/ MW, RS- RF Expt 2: Rats/ L-IML, MW, RS- RF	RS- FR2 region No details given	Spatial DNMS RAM SRL	Pre-op Pre-op Post-op	MW- severe RS- moderate MW, RS- moderate L-IML- severe L-IML- severe
1996	Young, Stevens, Converse & Mair	Expt 1: Rats/ L-IML, MD, Fx- RF Expt 2: Rats/MW, H, L-IML- RF Expt 3: Rats/L-IML, MW- RF	No details given No details given No details given	Spatial DNMS RAM Spatial DNMS Spatial DMS	Pre-op Post-op Pre-op Pre-op	MD, Fx- slight L-IML- moderate MD- slight Fx, L-IML- moderate MW, L-IML- moderate H- none L-IML, MW- moderate
1995	Aggleton, Neave, Nagle & Hunt	Rats/AT, MM-NMDA, Fx- RF	AT- LD, PV, Re, PT, nmd, Fx MM- supramammillary nucleus Fx- rostral H, fimbria, CC, cingulum bundle, AT	T-maze spatial forced alternation Continuous alternation SOR	Post-op Post-op Post-op	MM- moderate AT, Fx- severe AT, MM- moderate Fx- severe None

Table 1.1 continued

Year	Authors	Lesion (species/areas-method)	Extra damage	Tasks	Training	Deficits (none, slight, moderate, severe)
1993	Aggleton & Sahgal	Expt 1: Rats/AT, MM-NMDA, Fx- RF	No details given	Spatial DNMS	Pre-op	AT, Fx- moderate MM- none
		Expt 2: Rats/H- aspiration, Fx-RF	No details given	Spatial DNMS	Pre-op	H, Fx- moderate
1992	Mair & Lacourse	Expt 1: Rats/L-IML, midline thalamus, MM, midline + MM- RF	No details given	Spatial DNMS Open field behaviour	Pre-op Post-op	L-IML- severe L-IML- slightly more active, much fewer rearing responses
		Expt 2: Rats/ Fx- RF	No details given	Spatial DNMS	Pre-op	Fx- moderate
1992	Mair, Robinson, Koger, Fox & Zhang	Expt 1: Rats/ Medial, Lateral-NMDA	No details given	Spatial DNMS	Pre-op	None
		Expt 2: Rats/ L-IML, Anterior L-IML, posterior L-IML- RF	No details given	Spatial DNMS	Pre-op	L-IML- moderate Anterior, posterior- none
1990a	Stokes & Best	Rats/ MD- ELEC	No details given	RAM (working and reference)	Post-op	MD- moderate (both working and reference)
1990b	Stokes & Best	Rats/ MD- IBO	AD, CM, Ha, LD, PT, PV, intermediodorsal nuclei	RAM	Post-op	MD- severe
1989	Berracochea, Jaffard & Jarrard	Rats/AT, MD- IBO	No details given	T-maze temporal alternation RAM Spatial SRL	Post-op Post-op Post-op	MD- moderate AT- none None None
1989	Sutherland & Rodriguez	Rats/ AT- ELEC	No details given	Morris water maze acquisition Morris water maze retention	Post-op Pre-op	AT- moderate None

Table 1.1 continued

Year	Authors	Lesion (species/areas-method)	Extra damage	Tasks	Training	Deficits (none, slight, moderate, severe)
1988	Stokes & Best	Rats/MD- ELEC	Ha, PV, PT, Re, SM, PF, pretectal area	RAM- working memory	Pre-op	MD- severe
1983	Aggleton & Mishkin	Monkeys/MD-excision	AT, AM, Re, PT, nmd, centrum medianum, PF, MM tract, Cg, Fx, SM, cell loss in MM	DNMS	Pre-op	Moderate, delay depend
				Visual pattern discrimination	Post-op	None
				Spatial delayed response	Post-op	None except for subject with large nmd lesion

Note: for ease of comparison all tasks (spatial and non-spatial) in each study are listed

Abbreviations:

Deficits: none= no deficit; moderate= some deficit, but some recovery over time/ performance not quite at chance levels; severe= large deficit, performance at chance levels, no recovery over time.

AD- anterodorsal nuclei; AM- anteromedial nuclei; AT- anterior thalamic nuclei; AV- anteroventral nuclei; CC- corpus callosum; Cg- cingulate gyrus; CgC- cingulate cortex; CL- centrolateral nuclei; CM- centromedial nuclei; DG- dentate gyrus; DMS- delayed match to sample; DNMS- delayed non-matching to sample; EC- entorhinal cortex; EL- electrolytic; Fx- fornix; H- hippocampus (dH=dorsal, vH=ventral); Ha- habenular nuclei; IAM- interanteromedial nucleus; Ib- Ibotenic Acid
IL- intralaminar nuclei; IT- inferotemporal cortex; LD- laterodorsal nuclei; L-IML- lateral internal medullary lamina; LT- lateral thalamic aggregate; MD- mediodorsal nuclei; mid= midline; MM- mamillary bodies; MT- posterior thalamic aggregate; MW- medial wall of the prefrontal cortex; nmd- nucleus medialis dorsalis; NMDA- N-methyl-D-aspartic acid; PC- paracentral nuclei; PF- parafiscular nuclei; PFC- prefrontal cortex; Po- posterior nuclei; PPC- posterior parietal cortex; PRC- perirhinal cortex; PT- paratenial nuclei; PV- paraventricular nuclei; RAM- radial arm maze; Re- reuniens nuclei; RF- radiofrequency; Rh- rhomboid nuclei; RSC- retrosplenial cortex; SM- stria medularis; SOR- spontaneous object recognition; SRL- serial reversal learning; VL- ventrolateral nuclei; VM- ventromedial nuclei

1.5 The role of the thalamic nuclei in odour memory

Table 1.2 (pp26-27) provides a summary of studies of medial thalamic lesions and tasks involving an odour attribute. Considerably fewer studies have examined odour memory than spatial memory and these have focused on the MD, L-IML and IL.

1.5.1 The AT region

No study listed in Table 1.2 has examined the role of the AT in odour learning and memory. As mentioned previously, the AT is assumed to be part of an extended hippocampal system (Aggleton & Brown, 1999). Although there is still some disagreement over the role of the hippocampus in non-spatial memory, one recent experiment demonstrated that hippocampal lesions did not impair performance on an odour-odour association task (Alvarez et al., 2002). A previous study has shown that hippocampal lesions impair performance in the odour-place paired associate task used in the current experiment (Gilbert & Kesner, 2002). However, the impairment is probably due to the spatial component of the task, given the large amount of evidence for the role of the hippocampus in spatial memory, including the finding that hippocampal lesions do not impair performance on paired-associate tasks that do not involve a spatial component (Gilbert & Kesner, 2002). Based on this evidence, it is unlikely that the AT is involved in odour learning and memory when there is no spatial component. However this prediction remains to be tested.

1.5.2 The MT region

The MD is thought to be involved in odour memory because of its role in an olfactory thalamic-neocortical projection pathway. Cells in layer 3 of the olfactory cortex project to two thalamic areas: the central segment of the MD and the anterior segment of the submedial nucleus. These thalamic areas then project to the agranular insular and lateral and ventrolateral orbital areas of the prefrontal cortex (Groenewegen, 1988). Because of its connections to olfactory areas, then, it is suggested that the MD may be involved in odour memory and learning.

Eichenbaum, Shedlack and Eckmann (1980) presented one of the first studies that identified the MD as being involved in odour memory. Rats were trained in a go/no go odour discrimination task, odour detection and odour threshold tests. Although rats with MD lesions performed similarly to controls in the odour detection and odour threshold tests, they were significantly slower to learn

the go/no go odour discrimination task. The extent of the deficit on the odour discrimination task depended on both the similarity and novelty of the odours being discriminated and the interaction between these two variables. They also demonstrated that lesions to the lateral frontal cortex (olfactory projection area of the MD) impaired odour discrimination significantly more than lesions to the medial frontal cortex (a non-olfactory projection area of the MD), suggesting that odour memory may rely on selective connections between the MD and the lateral frontal cortex.

However, other studies have suggested that the MD may not be involved in odour discrimination and instead is involved in odour serial reversal learning (McBride & Slotnick, 1997; Slotnick & Kaneko, 1981). For example, McBride and Slotnick (1997) used an asymmetrical lesion paradigm where rats received either bilateral MD lesions, unilateral MD lesions plus contralateral olfactory bulbectomy and transection of the anterior commissure, or unilateral MD lesions plus contralateral lesions of the frontal cortex. Although no group was impaired on an odour discrimination task, rats with contralateral lesions to one of the MD olfactory projection areas performed as poorly as rats with bilateral MD lesions when required to learn the reversal of that task.

It is unclear why the serial reversal odour task is particularly sensitive to MD damage. It has been suggested that the relative complexity or high cognitive load of the task compared to simple odour detection or discrimination tasks may make it sensitive to MD damage (McBride & Slotnick, 1997). However, other studies have used complex olfactory learning tasks that are not sensitive to MD damage. For example, Zhang, Burk, Glode & Mair (1998) reported that large MD lesions had no effect on learning in an olfactory go/no go delayed non-match to sample (DNMS) task. Although the DNMS task is relatively complex, MD lesions did not impair performance, suggesting that it is not complexity *per se* that makes the serial reversal odour task sensitive to MD damage. Ray and Price (1992, cited by McBride and Slotnick, 1997) have suggested that the factor that makes serial reversal odour learning sensitive to MD damage is not complexity, but rather the requirement that animals withhold previously rewarded responses. They speculate that the MD is part of a pallidothalamic system involved in, specifically, the suppression of previously rewarded responses that are now unrewarded. In support of this, they cite evidence that lesions to other parts of this system, including the ventral pallidum, ventral striatum and medial prefrontal cortex, produce deficits in odour reversal learning similar to those seen after MD lesions (Ferry, Lu, & Price, 2000).

1.5.3 The LT region

Similar to studies examining the MT region, few studies have examined the role of the LT region in odour learning and memory. Two studies listed in Table 1.2 have examined the role of the LT region in odour memory, one focusing on two lesion targets (L-IML and IL), and the other focusing solely on the L-IML. Both studies reported impairments in olfactory continuous DNMS after L-IML lesions, although basic odour discrimination learning was unaffected (Koger & Mair, 1994; Zhang et al., 1998). Zhang, Burk, Glode and Mair (1998) also reported a more severe olfactory continuous DNMS impairment after lesions to an IL aggregate consisting of the paracentral, centrolateral and central medial nuclei.

There is some suggestion that rather than reflecting a specific impairment in odour memory, the impairments after lesions to the LT region may be the result of a more general impairment in learning. For example, IL and L-IML lesions produce deficits in a range of spatial tasks, including spatial DMS, spatial serial reversal learning, various radial arm maze tasks, and the Morris water maze task (Mair et al., 1998). The spatial deficits reported by Mair et al were also delay independent- the animals performed as poorly at short delays as they did at long delays. It is important to note, then, that the authors interpreted these results as evidence that IL lesions cause a general deficit in learning that is not tied to any specific attribute.

Table 1.2. Studies using thalamic lesions and behavioural tasks involving an odour memory component

Year	Authors	Lesion (species/areas-method)	Extra damage	Tasks	Training	Deficits (none, slight, moderate, severe)
1998	Zhang, Burk, Glode & Mair	Expt 1: Rats/L-IML, MD, PC+CL, EC, PY,-NMDA	Expt 1: No exact details given	Expt 1: Olfactory continuous DNMS	Pre-op	PY- severe L-IML- moderate
				Retention interval manipulation	Post-op	L-IML, MD- slight PY- moderate
				Stimulus set size manipulations	Post-op	None
				Nonodourised DNMS	Post-op	None
				Odour discrimination	Post-op	L-IML- slight
		Expt 2: Rats/IL (PC+CL+CM), MD-NMDA	Expt 2: IL- MD, LD, VM MD- none reported	Expt 2: Olfactory continuous DNMS	Pre-op	MD- slight IL- severe
				Odour discrimination	Post-op	None
1997	McBride & Slotnick	Rats/unilateral MD-EL + OB- excision; unilateral MD- ELEC + OB + AC-excision; unilateral MD- ELEC + FC- excision; bilateral MD- ELEC	No details given	Odour detection	Pre-op	MT+OB+AC-moderate
				Odour discrimination	Post-op	None
				Odour SRL	Post-op	MD+OB+AC- severe Bilateral MD, MD+FC- moderate MD+OB- slight

Table 1.2 continued

Year	Authors	Lesion (species/areas-method)	Extra damage	Tasks	Training	Deficits (none, slight, moderate, severe)
1994	Koger & Mair	Rats/L-IML, MW, RS-RF	L-IML- Ha, CM MW, RS- no details given	Olfactory continuous DNMS	Pre-op	L-IML- severe MW, RS- moderate
				Stimulus set size manipulation	Post-op	L-IML- severe MW, RS- none
				Retention interval manipulation	Post-op	Did not influence group differences
				Non-odourised DNMS	Post-op	None
				Odour discrimination	Post-op	None
1981	Slotnick & Kaneko	Rats/MD large (total), MD small (subtotal), amygdala- method not stated	No details given	Olfactory SRL	Post-op	MD large- severe
				Odour discrimination		None
1980	Eichenbaum, Shedlack & Eckmann	Rats/MD- RF, MW, RS, FP- excision	MD- PV, PT, SM, Ha, H MW- none FP- rhinal cortex RS- dorsolateral cortex	Odour discrimination	Pre-op	MD, FP- moderate, esp when odours similar MW- slight RS- severe
				Odour detection and threshold tests	Post-op	None

Note: for ease of comparison, all tasks in each study are listed

See Table 1.1 (page 22) for abbreviations

1.6 The role of the thalamic nuclei in spontaneous object recognition

Table 1.3 (pp29-31) provides a summary of studies using spontaneous object recognition tasks with medial thalamic lesions. Thus far, no study has found a deficit in spontaneous object recognition after thalamic lesions, at least in more basic recognition tasks. Most of the studies listed in Table 1.3 have focused on the AT. For example, Moran and Dalrymple-Alford (2003) found that AT lesions had no effect on spontaneous object recognition, even after delays of up to 40 minutes. Two other studies have failed to find an effect of AT lesions on spontaneous object recognition (Aggleton et al., 1995; Warburton & Aggleton, 1999).

Two of the studies listed in Table 1.3 have focused on the MD and LT. Hunt and Aggleton (1998) reported that MD lesions did not affect spontaneous object recognition. Mitchell & Dalrymple-Alford (2005) reported that neither AT, MT nor LT lesions had any effect on spontaneous object recognition, but there was some indication that MT and LT lesions impaired temporal memory for the order of presentation of objects. Wilton et al (2001) also reported no spontaneous object recognition deficit, but reported a deficit in object-in-place recognition following combined AD and LD lesions.

Table 1.3. Studies using thalamic lesions and spontaneous object recognition tasks

Year	Authors	Lesion (species/areas-method)	Extra damage	Tasks	Training	Deficits (none, slight, moderate, severe)
2005	Mitchell & Dalrymple-Alford	Rats/AT, MT, LT-NMDA	AT- MD, IL MT- AM, AV, AD, IL LT- AM, AV, AD	RAM- working and reference	Pre-op	AT- severe, both working and reference
				Reward magnitude go-no go	Post-op	MT- severe
				Temporal order (objects)	Post-op	MT, LT- severe
				SOR familiarity/novelty	Post-op	None
				SOR temporal order	Post-op	MT, LT- moderate
2004	Ridley, Baker, Mills, Green & Cummings	Monkey/unilateral AT- NMDA then second surgery with unilateral IT- excision (order of AT/IT counterbalanced)	AT- MD, ventroanterior thalamic nucleus, MM reduced IT- PRC	Simple object discrim	Pre-op1	None
				Successive object discrim	Post-op2	None
				Spatial discrimination	Post-op2	Moderate
				Visuospatial task	Pre-op2, post-op1	AT, IT- None AT+IT- moderate
				Spatiovisual task	Post-op2	AT+IT- Severe
				Visuovisual task	Post-op2	AT+IT- Severe
				Background spatial task	Post-op2	None
2003	Moran & Dalrymple-Alford	Rats/ AT, PRC-NMDA	AT- LD, PC, CL, CM, PT, IAM, Rh, MD, VL, reticular PRC- CA1, temporal cortex area 3, ER, postrhinal	RAM	Post-op	AT- severe PRC- none
				SOR	Post-op	None
				Elemental cue learning	Post-op	PRC- slight AT- none
				Configural cue learning	Post-op	PRC- moderate AT- none

Table 1.3 continued

Year	Authors	Lesion (species/areas-method)	Extra damage	Tasks	Training	Deficits (none, slight, moderate, severe)
2001	Wilton, Baird, Muir, Honey & Aggleton	Rats/AD+LD-NMDA	AV, AM, DG	T-maze spatial forced alternation	Post-op	AD+LD- severe
				Water maze beacon	Post-op	AD+LD- severe
				Object-in-place	Post-op	AD+LD- moderate
				SOR	Post-op	None
1999	Warburton & Aggleton	Rats/AT, AT+MD-NMDA, Fx- RF	AT- Re, LD, nmd, DG, MM	SOR	Post-op	No group effect
			AT+MD- LD, Re, nmd, MM	Morris water maze & probe test	Post-op	Fx- moderate AT, AT+MD- severe Probe test- no group effect
			Fx- AV, AD, S, CC			
				T-maze spatial forced alternation	Post-op	Fx, AT, AT+MD- moderate
1998	Hunt & Aggleton	Rats/MD- NMDA	LD, AD, AV, mid nuclei, PV, PT, DG, needle tract in H	RAM- working memory	Post-op	MD- slight-moderate
				RAM- reference memory	Post-op	MD- severe when maze rotated
				T-maze spatial forced alternation	Post-op	No group effect
				SOR	Post-op	No group effect
1998	Savage, Castillo, Langlais	Rats/L-IML- RF, IL+ (intralaminar + midline)- IBO	L-IML- AD, AM, mid nuclei, MD, Rh, gelatinosus nuclei	Successive object discrimination	Pre-op Post-op	No group effect No group effect
				Morris water maze	Post-op	IL+- moderate
			IL+- AT, MD, Rh, gelatinosus nuclei, Po, VL, MM	Morris Probe task	Post-op	IL+- moderate
				Repeated acquisition	Post-op	IL+- moderate
				Acoustic startle	Post-op	No group effect

Table 1.3 continued

Year	Authors	Lesion (species/areas-method)	Extra damage	Tasks	Training	Deficits (none, slight, moderate, severe)
1995	Aggleton, Neave, Nagle & Hunt	Rats/AT, MM-NMDA, Fx- RF	AT- LD, PV, Re, PT, nmd, Fx	T-maze spatial forced alternation	Post-op	MM- moderate AT, Fx- severe
			MM- supramammillary nucleus	Continuous alternation	Post-op	AT, MM- moderate Fx- severe
			Fx- H, fimbria, CC, cingulum bundle, AT	SOR	Post-op	No group effect

Note: for ease of comparison, all tasks in each study are listed

See Table 1.1 (page 22) for abbreviations

1.7 Issues regarding the location, method, specificity and analysis of lesions

Much of the Introduction thus far has suggested that a significant methodological issue even in experimental studies of thalamic lesions is the location, size, method and specificity of the lesions produced. As mentioned previously, even small encroachments of lesions into other thalamic areas may have contributed to inconsistent experimental results in previous studies (see Section 1.4.2).

An issue that is relevant to lesions to all areas (including AT, MT and LT) is the method used to produce the lesion. Several methods are available, including excitotoxic (NMDA, ibotenic acid), radiofrequency, electrolytic and excision or ablation (more commonly used in large animals such as monkeys). While excitotoxic lesions destroy only cell bodies, radiofrequency and electrolytic lesions also destroy fibres of passage. Thus, excitotoxic lesions are more often used in areas with a high density of cell bodies, such as the AT or MD, whereas radiofrequency or electrolytic lesions are more often used in fibre passage areas such as the L-IML and fornix.

There is also considerable variation between studies in the intentional damage included in, say, 'MD' or 'IL' lesions. Table 1.4 (pp34-38) gives a summary of the specificity of lesions in studies using thalamic lesions in rats. For example, an L-IML lesion aims to damage only the L-IML fibres in some cases (Burk & Mair, 1998; Mumby et al., 1999) but others also included intentional damage to the CL (Savage et al., 1997) or even the AM (Savage et al., 1998). The AM, of course, is more commonly included as part of the AT lesion. MT lesions typically target only the MD (Burk & Mair, 1998; Hunt & Aggleton, 1998), but one study has also explicitly included the IMD (Mitchell & Dalrymple-Alford, 2005).

There is also variation between studies in the size and location of unintentional damage to other structures. Because of the small size and close proximity of regions within the thalamus, lesions to one area inevitably produce damage to adjacent areas. For example, AT lesions typically also damage surrounding tissue in the LD (Mair et al., 2003; Ward-Robinson et al., 2002), MD (Mair et al., 2003) and IL (Warburton et al., 1999). MD lesions have been reported to encroach on the AT (Hunt & Aggleton, 1998) and L-IML (Burk & Mair, 1998), while lesions that include the IL have produced unintentional damage to the AT (Savage et al., 1998) and MD (Mair et al., 1998; Zhang et al., 1998), and L-IML lesions have damaged the AT (Savage et al., 1997), and MD (Burk & Mair, 1998; Savage et al., 1997). Previous research using thalamic lesions has typically provided only qualitative, rather than quantitative, data regarding the damage caused by lesions. Given the variability in the location and extent of both intentional and unintentional damage, a quantitative,

rather than qualitative, analysis of lesion location and size would be valuable. However Table 1.4 shows that, while many studies provide simple qualitative histological data such as lists of areas that were damaged and pictures of "typical" or "representative" lesions, very few studies have provided a detailed quantitative analysis of the damage produced by their lesions. One important exception is the recent study from Mitchell and Dalrymple-Alford (2005) that made explicit attempts to minimise unintentional damage, reduce overlap between lesions, and used a detailed quantitative analysis of lesion damage. Although the lesions from this study produced unintentional damage to the non-target aggregates, this damage was typically relatively minor, as was damage to other structures not included in the aggregates.

Aside from this, only one other study has reported a detailed quantitative analysis of lesion damage (Byatt & Dalrymple-Alford, 1996). One other study has provided a semi-quantitative analysis, where intentional and unintentional damage was coded with '1', '2', or '3' according to the estimated size of the damage (Moran & Dalrymple-Alford, 2003). Two other studies (Savage et al., 1998; Savage et al., 1997) provided an analysis of percentage damage to the mammillary bodies only. While several other studies have examined the relationship between lesion size and task performance, this is typically done by ranking the lesions in order according to the estimated amount of damage to the target areas, rather than quantifying the amount of damage to both target and non-target structures in each lesion (see, for example, McBride & Slotnick, 1997; Zhang et al., 1998).

The current study attempted to use small, highly localised excitotoxic lesions and perform a detailed quantitative analysis of the percentage damage to a number of thalamic regions caused by these lesions. Excitotoxic lesions were used to avoid unintentional damage to fibre pathways in the area. The quantitative analysis of lesion damage now used at the University of Canterbury is a novel, but potentially valuable, tool for histological analysis.

Table 1.4. Specificity and analysis of lesions in studies using thalamic lesions in rats (only studies listed in Tables 1-3 are included)

Year	Authors	Lesion (method)	●=target damage ○=unintended damage -=not damaged N=no details														Ql	Qn	Lg/ Sml	Typ	Cor
			AM	AV	AD	MD	IL	l-IML	MM	Re	LD	CM	CL	PT	PC	Other					
2005	Mitchell & Dalrymple-Alford	AT (NMDA)	●	●	●	○	○	N	N	○	○	○	○	○	○	○ IAM	Y	Y	Y	-	Y
		MT (NMDA)	○	○	○	●	○	N	N	○#	-	○	○	○	○	●IMD ○ PV ○ IAM					
		LT (NMDA)	○	○	○	●	●	N	N	○	○	●	●	○	○	○ IAM					
2003	Mair, Burk & Porter	AT (NMDA)	●	●	●	○	○	-	-	-	○	-	○	-	-	-	Y	-	Y	Y	-
2003	Moran & Dalrymple-Alford	AT (NMDA)	●	●	●	○	-	-	-	-	○	○	○	○	○	○IAM ○Rh ○VL ○Ret	Y	Y	Y	-	Y
2002	Van Groen, Kadish & Wyss	LD (IBO)	-	-	-	-	-	-	-	-	●	-	-	-	-	-	Y	-	Y	-	-
		LD+AD+AV (IBO)	-	○	○	-	-	-	-	-	●	-	-	-	-	-					
2002	Ward-Robinson, Wilton, Muir, Honey, Vann & Aggleton	AT (NMDA)	●	●	●	○	-	-	-	○	○	-	-	-	-	○mid ○DG	Y	-	Y	-	-
2001	Alexinsky	AT (IBO)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	-	-	Y	-	-
		MD (IBO)	N	N	N	N	N	N	N	N	N	N	N	N	N	N					
2001	Gaffan, Bannerman, Warburton & Aggleton	AT (NMDA)	●	●	●	○	-	-	-	-	-	-	-	-	-	-	Y	-	Y	-	-
2001	Wilton, Baird, Muir, Honey & Aggleton	AD+LD (NMDA)	○	○	●	-	-	-	-	-	●	-	-	-	-	○DG	Y	-	Y	-	-
1999	Mumby, Cameli, Glenn	L-IML (ELEC)	○	-	-	○	-	●	-	-	-	○	○	-	○	○VL	Y	-	Y	-	-

Table 1.4 continued

Year	Authors	Lesion (method)	●=target damage ○=unintended damage -=not damaged N=no details													Ql	Qn	Lg/ Sml	Typ	Cor
			AM	AV	AD	MD	IL	l-IML	MM	Re	LD	CM	CL	PT	PC	Other					
1999	Sziklas & Petrides	AT (ELEC)	●	●	●	○	-	-	-	-	○	-	-	○	○	○PV ○IAM	Y	-	Y	-	-
1999	Warburton & Aggleton	AT (NMDA)	●	●	●	-	-	-	○	○	○	-	-	-	-	○DG	Y	-	Y	-	-
		AT+MD (NMDA)	●	●	●	●	-	-	○	○	○	-	-	-	-	-					
1999	Warburton, Morgan, Baird, Muir & Aggleton	AT (NMDA)	●	●	●	○	○	-	-	○	○	○	○	○	○	○mid ○ret ○DG	Y	-	Y	Y	-
1998	Burk & Mair	MD (NMDA)	-	-	-	●	-	○	-	-	-	-	-	-	-	-	Y	-	Y	Y	-
		IL (NMDA)	-	-	-	○	●	-	-	-	○	○	●	-	●	○VM ○lpn					
		L-IML (NMDA)	-	-	-	○	-	●	-	-	○	-	○	-	○	○VM					
1998	Hunt & Aggleton	MD (NMDA)	-	○	○	●	-	-	-	-	○	-	-	○	-	○mid ○PV ○DG	Y	-	Y	Y	Y
1998	Mair, Burk & Porter	IL (NMDA)	-	-	-	○	●	-	-	-	○	-	-	-	-	○VM ○lpn	Y	-	Y	-	-
1998	Savage, Castillo, Langlais	L-IML (RF)	●	-	○	○		●	-	-	-	●	●	-	●	●PF ●IAM ○Rh ○gel	Y	Y (*)	Y	-	-
		IL + mid (IBO)	○	○	○	○	●	-	○	-	-	-	-	-	-	●mid ○Rh ○gel ○VL					

Table 1.4 continued

Year	Authors	Lesion (method)	●=target damage ○=unintended damage -=not damaged N=no details														Ql	Qn	Lg/ Sml	Typ	Cor
			AM	AV	AD	MD	IL	l-IML	MM	Re	LD	CM	CL	PT	PC	Other					
1998	Zhang, Burk, Glode & Mair	L-IML (NMDA)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	-	Y	-	Y
		MT (NMDA)	N	N	N	N	N	N	N	N	N	N	N	N	N	N					
		PC+CL (NMDA)	N	N	N	N	N	N	N	N	N	N	N	N	N	N					
		IL (NMDA)	-	-	-	○	◐	-	-	-	○	-	-	-	-	○VM					
1997	McBride & Slotnick	MD (ELEC)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	-	Y	-	Y
1997	Savage, Sweet, Castillo & Langlais	L-IML (RF)	○	○	-	○	-	●	○	-	-	○	●	-	●	○Rh ○gel	Y	Y (*)	Y	-	-
1997	Warburton, Baird, Aggleton	AT (NMDA)	●	●	●	○	-	-	-	○	○	-	-	-	-	○ret ○DG	Y	-	Y	-	-
		AT+LD (NMDA)	●	●	●	○	-	-	-	○	●	-	-	-	-	-					
1996	Aggleton, Hunt, Nagle & Neave	AM (NMDA)	●	-	-	-	-	-	-	-	-	-	-	○	-	-	Y	-	Y	-	-
		AV+AD (NMDA)	○	●	●	-	-	-	-	-	-	-	-	-	-	-					
		AM+AV+AD (NMDA)	●	●	●	○	-	-	○	-	○	-	-	-	-	-					
1996	Byatt & Dalrymple-Alford	AM (RF)	●	○	○	-	-	-	-	-	-	○	-	○	-	○SM ○PV ○Rh	Y	Y	Y	Y	Y
		AV (RF)	○	●	○	-	-	-	-	○	-	-	-	-	-	○SM ○VL					
1996	Harrison & Mair	L-IML	N	N	N	N	N	N	N	N	N	N	N	N	N	N	-	-	-	Y	-

Table 1.4 continued

Year	Authors	Lesion (method)	●=target damage ○=unintended damage -=not damaged N=no details														Ql	Qn	Lg/ Sml	Typ	Cor
			AM	AV	AD	MD	IL	l-IML	MM	Re	LD	CM	CL	PT	PC	Other					
1996	Young, Stevens, Converse & Mair	L-IML (RF)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	-	-	Y	-	-
		MD (RF)	N	N	N	N	N	N	N	N	N	N	N	N	N	N					
1995	Aggleton, Neave, Nagle & Hunt	AT (NMDA)	●	●	●	-	-	-	-	○	○	-	-	○	-	○PV ○Fx ○nmd	Y	-	Y	Y	-
1994	Koger & Mair	L-IML (RF)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	-	-	-	Y	-
1993	Aggleton & Sahgal	AT (NMDA)	●	●	●	-	-	-	-	-	-	-	-	○	-	○PV	Y	-	Y	-	-
1992	Mair & Lacourse	L-IML- RF	N	N	N	N	N	N	N	N	N	N	N	N	N	N	-	-	-	Y	-
		Mid- RF	N	N	N	N	N	N	N	N	N	N	N	N	N	N					
1992	Mair, Robinson, Koger, Fox & Zhang	Medial (NMDA)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	-	-	-	Y	-
		Lateral (NMDA)	N	N	N	N	N	N	N	N	N	N	N	N	N	N					
		L-IML (posterior/anterior/all) (RF)	N	N	N	N	N	N	N	N	N	N	N	N	N	N					
1989	Sutherland & Rodriguez	AT (ELEC)	●	●	●	N	N	N	N	N	N	N	N	N	N	N	-	-	-	Y	-
1988	Stokes & Best	MD (ELEC)	-	-	-	●	-	-	-	-	-	-	-	○	-	○ Ha ○ PV ○ Re ○ SM ○ PF ○Prt	Y	-	Y	-	-
1985	Markowitsch, Kessler & Streicher	AT+MD (ELEC)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	-	-	-	Y	-

Table 1.4 continued

Year	Authors	Lesion (method)	•=target damage ○=unintended damage -=not damaged N=no details													Ql	Qn	Lg/ Sml	Typ	Cor
			AM	AV	AD	MD	IL	l-IML	MM	Re	LD	CM	CL	PT	PC	Other					
1981	Slotnick & Kaneko	MD large (total)-method not stated	N	N	N	N	N	N	N	N	N	N	N	N	N	N	-	-	-	Y	-
		MD small (subtotal)-method not stated	N	N	N	N	N	N	N	N	N	N	N	N	N	N					
1980	Eichenbaum, Shedlack & Eckmann	MD (RF)	-	-	-	•	-	-	-	-	-	-	-	○	-	○ PT ○ SM ○ Ha ○ H	Y	-	Y	Y	Y

* Quantitative analysis for mammillary body damage only

Extremely minor damage only

Abbreviations/notes:
Ql= qualitative analysis (descriptions of lesions, lists of damaged areas)
Qn= quantitative analysis (measurement of % damage or similar)
Lg/Sml= pictures/drawings of the largest and smallest lesions
Typ= picture/drawing of a 'typical', 'median' or 'representative' lesion
Cor= analysis of the correlation/relationship between lesion size and task performance

AD= anterodorsal nucleus; AM= anteromedial nucleus; AT= anterior thalamic aggregate; AV= anteroventral nucleus; Cg= cingulate gyrus; CL= centrolateral nucleus; CM= centromedial nucleus; DG= dentate gyrus; ELEC= electrolytic; Fx= fornix; Gel= gelatinosus nucleus; H= hippocampus; Ha= habenular nuclei; IAM= interanteromedial nucleus; IBO= ibotenic acid; IL= intralaminar nuclei; IMD= intermediodorsal nucleus; LD= laterodorsal nucleus; l-IML= lateral internal medullary lamina; LT= lateral thalamic aggregate; lpn= lateral posteior nucleus; MD= mediodorsal nucleus; mid= midline nuclei (unspecified); MM= mammillary bodies; MT= posterior thalamic aggregate; nmd= nucleus medialis dorsallis; NMDA= N-methyl-D-aspartic acid; PC= paracentral nuclei; PF= parafiscular nuclei; Prt= pretectal area; PT= paratenial nuclei; PV= paraventicular nuclei; Re= nucleus reuniens; Ret= reticular formation; RF= radiofrequency; Rh= rhomboideus nucleus; SM= stria medularis; VL= ventrolateral nuclei; VM= ventromedial nucleus

1.8 The experimental procedures employed in this study

1.8.1 Odour-place paired-associate task

The formation of an episodic memory requires not just that a number of attributes of an event are encoded, but that these attributes are bound together in a unique way. Hence, paired-associate learning ('pattern association') tasks are often regarded as measures of 'episodic-like' memory in non-human animals. Paired-associate tasks that involve a spatial component are thought to be particularly relevant to the study of episodic memory, because the attributes of an episodic memory are linked together in a spatial array (Aggleton & Pearce, 2001).

The primary purpose of the present study was to examine the effects of lesions to three aggregates of thalamic nuclei (AT, MT and LT) on an odour-place paired-associate learning task. It is important to note that the task is an acquisition task, thus all training occurs after surgery. The specific task used was employed previously to study the role of the hippocampus in paired-associate learning (Gilbert & Kesner, 2002, 2003), but has not been used to study the effects of thalamic lesions. While some studies have found that hippocampal lesions impair performance only when the pattern association involves a spatial component (Gilbert & Kesner, 2002), there is evidence that the hippocampus may also be involved in non-spatial memory. Hippocampal lesions can impair memory for odour-odour associations in a social transmission of food preference paradigm independent of spatial context (Alvarez et al., 2002) and parahippocampal lesions can impair memory for odour-odour paired-associates with no spatial component (Bunsey & Eichenbaum, 1993). However, a recent study demonstrated that hippocampal lesions had no effect on a one-trial odour-reward association with no spatial component (Wood, Agster, & Eichenbaum, 2004). While the role of the hippocampus, and indeed that AT, in non-spatial tasks is unclear, there is considerable evidence that these structures are involved in memory that does include a spatial component. Given that the hippocampus is presumed to be part of an extended hippocampal system that also includes the AT (Aggleton & Brown, 1999), it would be expected that AT lesions would also impair performance on this odour-place paired associate task. The similarity of the effects of hippocampal and AT lesions on this task would provide further support for the existence of an extended hippocampal system. It is, however, also important to compare selective AT, MT and LT lesions in this task.

In addition to the standard odour-place paired-associate task, a set of probe trials were introduced into the current study to investigate the use of allocentric and egocentric spatial strategies. In the standard version of the task, it is assumed that an association is made between an odour and the spatial location in which it is presented. However, it is possible that the animals use a number of cues additional to, or instead of, spatial location to form the associations. These could include response (body turn) and direction (landmark) cues. To assess the extent to which these non-spatial cues were being used, the start position was shifted to the opposite side of the apparatus at the end of training on the standard odour-place task. In the probe task the use of any egocentric cues was now inappropriate, whereas animals could still solve the task by using allocentric cues. The examination of allocentric versus egocentric learning is important because previous evidence has suggested that AT lesions may affect these two types of learning differently. In the only previous study to examine paired-associate learning after thalamic lesions, Sziklas and Petrides (1999) demonstrated that AT lesions impair object-place paired-associate learning, but do not impair object-response paired associate learning. Some inconsistencies in research results may be due to the use of egocentric strategies instead of the presumed allocentric strategies (Berracochea et al., 1989).

1.8.2 Simple odour discrimination and simple place discrimination tasks

Rats were also tested on a go-no go discrimination task for either the two odours or the two spatial locations used in the odour-place paired associate task. The aim of these simple discrimination tasks was to determine whether impaired performance on the odour-place paired associate task was due to an inability to discriminate between the odours or places used or an inability to inhibit digging responses. Because the discrimination tasks involved only simple discrimination, with no pattern association processing, they served as a control for the odour-place paired-associate task. Previous studies (Gilbert & Kesner, 2002; 2003) have shown that hippocampal lesions do not impair performance on either odour or spatial discrimination tasks.

1.8.3 Spontaneous object recognition tasks

A common method used to study the memory processing of objects is the spontaneous object recognition task. Spontaneous object recognition tasks exploit rats' natural tendency to explore novel objects more than familiar objects. Because the task requires no pretraining, it is commonly

regarded as providing a closer analogy to recognition tasks used in humans (Dix & Aggleton, 1999). The lack of pretraining also avoids several possible confounds, such as the failure to apply a learned rule or a change in an animal's desire for food reinforcement, that are present in other object recognition tasks such as DMS and DNMS (Dix & Aggleton, 1999).

This current study also used spontaneous object recognition procedures to assess memory for familiar and novel objects and object-defined spatial locations. In the standard task, two objects are presented in an open field and the rat is given a period of time in which to become familiar with them. The objects are then removed and replaced with a duplicate of one of the objects and a novel object. It is presumed that if rats recognise the presented object, they will spend longer exploring the novel object. If they have no memory of the previously presented object, it is presumed they will explore both objects equally. It has been reported that performance in the standard spontaneous object recognition task is unaffected by lesions to the AT (Moran & Dalrymple-Alford, 2003; Warburton & Aggleton, 1999), MD (Hunt & Aggleton, 1998; Mitchell & Dalrymple-Alford, 2005) and LT (Mitchell & Dalrymple-Alford, 2005). The spontaneous object recognition task employed in this study was an extension of the basic spontaneous object recognition task, based on similar techniques developed by Poucet (Save, Poucet, Foreman, & Buhot, 1992). Poucet's procedure allows, however, the use of both novel objects and novel spatial locations for familiar objects. In addition, the procedure also includes novel associations of familiar objects and places, where a familiar object is moved to a familiar location. Using this modified version of the task, Save, Poucet, Foreman and Buhot (1992) examined the effects of lesions to the hippocampus, anterior parietal cortex (APC) and posterior parietal cortex (PPC) on spontaneous object recognition. They found that all three groups preferentially explored novel objects at a level similar to that of control animals. However, when a familiar object was displaced to a different location, hippocampal and PPC animals did not show increased exploration. APC animals showed increased exploration of both displaced and non-displaced objects and control animals showed increased exploration of the displaced objects only. While no study thus far has found that AT, MT or LT lesions impair spontaneous object recognition, there is evidence that the AT, and possibly the MT and LT, are involved in spatial memory. Therefore it was expected that, although all groups would show increased exploration of novel objects, the AT group, and perhaps MT and LT groups, may not show increased exploration of the displaced objects.

1.9 Aims of the current study

The major aim of the current study was to directly compare the effects of highly selective lesions to the AT, MT and LT regions on Kesner's (Gilbert & Kesner, 2002, 2003) odour-place paired associate task. No previous study has compared the effects of lesions to the AT, MT and LT on a paired-associate learning task. This is surprising because paired-associate tasks are one of the few 'episodic-like' memory tasks that can be used with animal models. A strength of the current study was the selectivity and detailed quantitative analysis of the lesions produced. Although there is evidence that variability in the size and location of lesions can affect experimental results, few studies have attempted a quantitative analysis of the damage caused by their lesions.

The other aims of the current study were to examine the effects of lesions to the AT, MT and LT on odour discrimination and place discrimination, and on Poucet's (Save et al., 1992) version of a series of spontaneous object recognition tasks. The odour discrimination and place discrimination tasks were included to examine whether impaired performance on the odour-place association task was due to an inability to discriminate between odours or places or an inability to withhold responses. It is important to rule out these possibilities to ensure that any deficits seen on the odour-place association task are in fact deficits in odour-place association, rather than an impairment in the basic procedural or discrimination requirements of the task. Spontaneous object recognition tasks have the advantage of avoiding a number of possible confounds of trained object recognition tasks and being a closer analogy of human recognition tests because they require no pretraining. The current study used a version that allowed an examination of memory for objects and also object-place associations in an efficient manner (single session).

While a common view of diencephalic amnesia is that a single diencephalic region is responsible for the memory deficit, an alternative view is that no single diencephalic region is responsible for the memory deficit. Instead, different diencephalic regions may contribute to memory in subtly different ways, which has been supported by recent research on selective AT, MT and LT lesions. The current study sought further support for this view. It was predicted that AT lesions would impair performance on the odour-place association task and impair detection of changes in object-place associations in the spontaneous object recognition task, due to the spatial components of these tasks. Despite the MT being implicated in odour learning and memory, it was predicted that MT lesions would have no effect on the tasks in the current study, because deficits after MT lesions seem to be specific to odour serial reversal learning. Finally, it was predicted that

LT lesions would affect performance on all of the tasks, because evidence points to the LT possibly playing a general role in learning and memory that is not tied to any specific attribute.

2. Materials and Method

2.1 Subjects

Thirty-five naïve female PVGc Hooded rats were used as subjects. All rats were aged ten months old and weighed between 150 and 200g at the time of surgery. Rats were housed in groups of three or four and maintained on a 12 hour reversed light-dark cycle (off 8am to 8pm). All testing was conducted during the dark portion of the cycle. Subjects' weight was maintained at 80% to 85% of free-feeding body weight throughout the preoperative training, odour-place paired-associate task and simple discrimination tasks, although free access to food was available during the post-surgery recovery period and for several days prior to the spontaneous object recognition task. Water was freely available throughout the study. All procedures conformed to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Canterbury Animal Ethics Committee (see Appendix A for ethics committee approval).

2.2 Apparatus

A circular board was used for training in the odour-place paired-associate and discrimination tasks. The board was 119 cm in diameter × 3.5 cm thick and stood 65 cm above the floor. It was painted white and had a flat surface and no wall around the perimeter. The board was located in the same position in the same room for all training and testing. A start box (24 cm long×15cm wide×17cm high, painted black) was placed on the surface of the maze, with its rear wall adjacent to the outer edge of the apparatus (see Figure 2.1 (p45), position A). The start box contained a small black door that could be raised and lowered manually by the experimenter. A camera mounted to the roof above the board was used to record behavioural data. The maze was in a well-lit room with no windows. A chair and low white table, a beige curtain covering half of one wall, and pictures on the walls provided additional spatial cues to those provided by the room itself.

Rats were trained to dig in small black painted terracotta pots (6cm wide at the top × 6cm high) to retrieve a hidden food reward. At the bottom of each pot was a layer of Froot Loops covered with wire mesh. These inaccessible Froot Loops served to ensure that rats could not use food odours to distinguish the baited pots. The pots were attached to small wooden black painted platforms (15cm × 15cm) when on the board so that the rat could not move the pots or spill their contents onto

the board. Only one of these pots was on the board at any one time. During the odour-place paired-associate training and the probe testing, the pots appeared in one of two locations (C and E, Figure 2.1, this page), 67 cm apart, each equal distance from the centre of the board, 43.5 cm from the door of the start box placed at position A (Figure 2.1). During the simple spatial discrimination task, the pots appeared in the previously described locations, but during the simple odour discrimination task they appeared at location D directly in front of and 28 cm away from the start box (Figure 2.1).

During the probe trials, the start box appeared on the opposite side of the board which occurred on half of the trials (see Figure 2.1, position B). To ensure that the distance from start box to digging pots remained the same when the start box was shifted to the new location, the start box was moved back off the edge of the board and rested on a platform (68.5 cm high × 29 cm long × 20 cm wide) attached to the floor. The platform was coloured white.

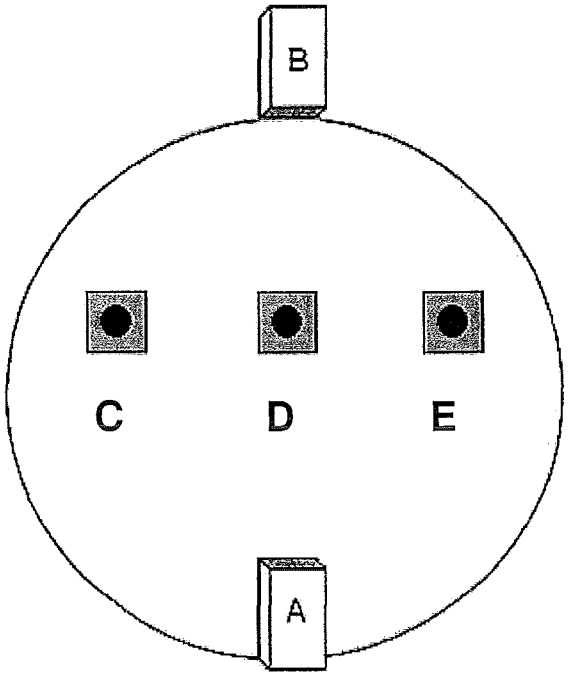


Figure 2.1 The board apparatus. Start box is shown in the original position (A) and the position used in the spatial probe trials (B). Locations of the digging pots on the odour-place paired-associate task and spatial discrimination task (C and E) and pretraining and odour discrimination task (D) are indicated by the filled squares. Note: only one digging pot was on the board at any one time.

A new circular board, again painted white, was used for the spontaneous object recognition task. The apparatus and procedures were based on those used previously by Poucet (Save et al, 1992), but the board used in the current study was slightly smaller than that used by Poucet and had transparent, rather than opaque, walls. The objects in the current study were placed so that all were clearly visible from the start position, which resulted in the locations of objects being slightly different to those used by Poucet. The board was located in the same position in the same room as for the odour-place paired-associate and simple discrimination tasks. The new board was also 119 cm in diameter × 3.5 cm thick and stood 65 cm above the floor. The surface of the apparatus was marked with a number of black lines to aid data coding (see Figure 2.2, p47). Attached to the perimeter of this board was a clear plastic wall, 30cm high and 2mm thick. The plastic was joined at four equally spaced points around the perimeter. At these joins a small piece of wood, 4 cm wide, was attached to the outside of the wall. The piece of wood ran from the bottom of the wall to 5cm below the top of the wall. On the surface of the board were 6 small screw holes to attach objects. The position of these holes is shown in Figure 2.2. A number of objects, each with a screw glued to their base, were attached to the surface of the board on certain trials. The objects used were duplicates of: a glass bottle (20.5 cm high and 4.5cm diameter at base); a plastic soap dispenser (16.5 cm high and 8.5 cm wide × 5 cm long at base); a glass vase (20.5 cm high and 7 cm long × 7 cm wide at base); a plastic bed leg (20 cm high and 4.5 cm diameter at base); a plastic painted monkey (10.5 cm high and 7.5 cm long × 7 cm wide at base); and a painted clay ornament (16 cm high and 5 cm wide × 8.5 cm long at base). These objects are shown in Figure 2.3 p47).

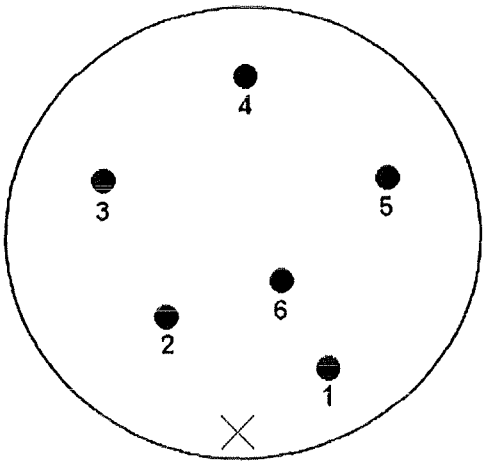


Figure 2.2. The spontaneous object recognition board. Locations where objects could be located are indicated by the numbers 1 to 6. The rat began each trial by being placed facing the edge of the board at '×'.

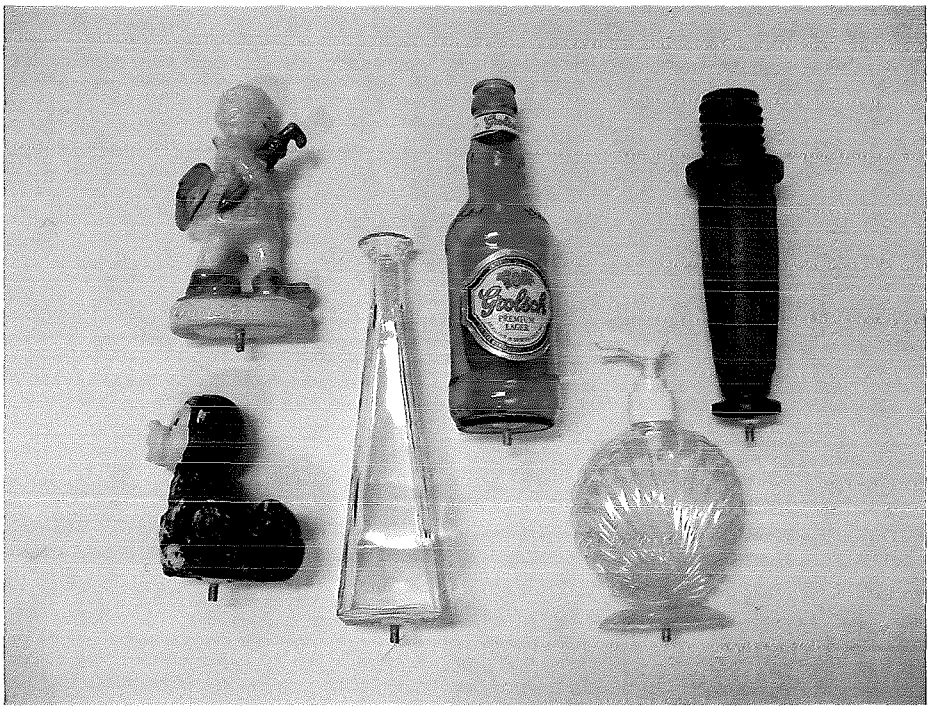


Figure 2.3. The objects used in the spontaneous object recognition task. From left to right: plastic monkey (bottom); ceramic ornament (top); glass vase; glass bottle; soap holder; plastic bedleg.

2.3 Surgical procedures

Rats were randomly assigned to the AT, MT, LT or sham lesion groups. Rats were anaesthetised 25 minutes after atropine (0.13 mg/ml, at a dose of 1.5 ml/kg IP) with sodium pentobarbitone (50 mg/ml, at a dose of 1.40 ml/kg IP). Stereotaxic co-ordinates for lesion placements were based on those used previously at the University of Canterbury (Mitchell & Dalrymple-Alford, 2005), after further verification and minor improvements in pilot work. Following surgery, each rat was given a 3 week recovery period before re-familiarisation to the board.

2.3.1 Anterior thalamic region (AT) lesions

Anaesthetized rats were placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga) with the incisor bar set 7.5 mm below the interaural line. This position was used to enable a vertical insertion of the needle whilst avoiding damage to the fornix. After being placed in the stereotaxic apparatus, but prior to incision, the shaved and cleansed scalp was locally anaesthetized with mepivicaine (2.0 mg/ml 0.2 ml subcutaneous under the scalp) and an injection of ketophen was administered (1.0 mg/ml, 0.1 ml subcutaneous, nape of neck). After craniotomy, microinfusions of 0.12 M NMDA (Sigma Chemicals, Australia) dissolved in phosphate buffer pH 7.20 were made via a 1-μl Hamilton syringe connected to a motorized infusion pump (Stoelting). The infusion needle was lowered very slowly to a given site, allowed to rest at situ for 30 seconds prior to infusion, left in situ for 3 min following the infusion to allow the NMDA to diffuse, and then slowly removed from the brain.

The anterior thalamic lesion consisted of two bilateral infusion sites, one aimed at the anteroventral nucleus (AV) and the other at the anteromedial nucleus (AM). To ensure lesion accuracy, all measures were taken from Bregma, but anterior-posterior (AP) co-ordinates in the horizontal plane were varied according to the exact Bregma to Lambda distance in each rat. Table 2.1 (p50) shows the Bregma-Lambda distances used and their corresponding co-ordinates for AT, MT and LT lesions. As shown, AP coordinates at the AV site ranged from -0.250 to -0.280 cm for AT lesions, 0.148 cm lateral to the midline (ML), and -0.555 cm ventral from dura (DV). At the AM site, AP coordinates ranged from -0.240 to -0.270 cm, and were 0.123 cm ML and -0.580 cm DV. The volume of NMDA infused at the AV site was 0.10 μl at a rate of 0.03 μl/min. The volume

infused at the AM site was 0.09μl at a rate of 0.03 μl/min. AT infusions were always performed in the same order: AV (for example) left; AV right; AM left; AM right.

2.3.2 Posterior medial thalamic region (MT) lesions

The basic procedure for MT lesions was the same for that used for AT lesions. The MT lesion consisted of two infusion sites, both centred at midline. For MT lesions, AP at the anterior site was -0.350 to -0.380 cm, and 0.16 μl NMDA was infused at a rate of 0.04 μl/min at -0.560 cm DV. At the posterior MT site, AP was -0.390 to -0.420 cm, with 0.18 μl NMDA infused at a rate of 0.04 μl/min at -0.570 cm DV. For MT lesions, the anterior infusion was always performed first, followed by the posterior infusion.

2.3.3 Lateral thalamic region (LT) lesions

The basic procedure for LT lesions was the same for that used for AT and MT lesions. The LT lesion consisted of three bilateral infusion sites. For the anterior LT site, AP was -0.345 to -0.375 cm, at 0.130 cm ML, and first a volume of 0.06 μl of NMDA was infused at a rate of 0.03 μl/min at -0.560 cm DV, followed by 0.05 μl infused at a rate of 0.03 μl/min at -0.600 cm DV. AP at the posterior LT site was -0.385 to -0.415 cm and was 0.130 cm ML, with 0.05 μl NMDA infused at a rate of 0.03 μl/min at -0.560 cm DV. The six infusions were always in the following order: anterior dorsal (for example) left; anterior ventral left; anterior dorsal right; anterior ventral right; posterior left; posterior right.

2.3.4 Sham lesion surgeries

Sham lesion controls also received surgery but no infusion. In most cases (N=6) the surgical procedure was identical to that used for lesion surgeries, except that a clean infusion needle was lowered to 0.300 cm above the lesion site (3 AT, 1 MT, 2 LT) and no solution was infused. The remaining animals (N=3) received the same general surgical procedure, except that following craniotomy the needle was not lowered into the brain.

Table 2.1. Lesion co-ordinates and related parameters for: individual Bregma-Lambda distances and corresponding AP co-ordinates for the AT, MT and LT lesions.

	AT		MT		LT		
	Anterior (AM)	Posterior (AV)	Anterior	Posterior	Anterior (two sites)		Posterior
B-L distance for co-ordinates							
0.60-0.61	-0.24	-0.25	-0.350	-0.390	-0.345		-0.385
0.62-0.63	-0.25	-0.26	-0.360	-0.400	-0.355		-0.395
0.64-0.66	-0.26	-0.27	-0.370	-0.410	-0.365		-0.405
0.67-0.68	-0.27	-0.28	-0.380	-0.420	-0.375		-0.415
0.69-0.70	-0.27	-0.28	-0.380	-0.420	-0.375		-0.415
0.71-0.72	-0.27	-0.28	-0.380	-0.420	-0.375		-0.415
ML	±0.123	±0.148	0.0	0.0	±0.130		±0.130
DV	-0.580	-0.555	-0.560	-0.570	-0.560	-0.600	-0.560
Volume μl	0.09	0.10	0.16	0.18	0.06	0.05	0.05
Rate μl/min	0.03	0.03	0.04	0.04	0.03	0.03	0.03

Abbreviations: AM= anteromedial site; AT= anterior thalamic aggregate; AV= anteroventral site; B-L= Bregma-Lambda; DV= dorsal-ventral distance from dura; LT= lateral thalamic aggregate; ML= medial-lateral distance from midline; MT= posteromedial thalamic aggregate.

2.4 Odour-place paired-associate task

2.4.1 Pre-operative familiarisation and training

During the first two weeks of training, rats were allowed free exploration of the board for approximately 10 minutes per day. Rats were placed on the board in home cage groups (3 or 4 rats) and 15 to 20 food rewards (pieces of Froot Loop cereal) were distributed across the surface for the rats to find. Following the first two weeks of training rats were shaped in a cage in the experimental room to dig in the small pots of sand in order to gain a food reward. Shaping began by placing the food reward on top of the sand for the rat to find. Food rewards were then gradually buried deeper in

the sand, until the rat dug for the food reward even when it was completely buried (approximately 2 cm beneath the surface).

Once rats were consistently digging in the sand to retrieve hidden food rewards they were shaped to dig for rewards on the board. Single rats were placed in the start box and a sand-filled pot on a platform was placed directly outside the start box door and the rat was allowed to locate and retrieve the buried food reward. The rat was then returned to the start box. This procedure was repeated 12 times per day, 5 days per week for 2 weeks, then every 2 to 3 days for the one or two weeks preceding surgery.

2.4.2 Re-familiarisation

Following a post-surgery recovery period of 3 weeks, rats were re-familiarised to the board. Rats were placed in the start box and a sand-filled pot was placed directly outside the start box, and familiarisation trials (12 per day for 2 days) were conducted as previously (see section 2.4.1).

2.4.3 Odour-place paired-associate training

Rats were trained on a go/no-go paired associate task in a similar fashion to that used by Gilbert and Kesner (2002, 2003). Powdered odours were thoroughly mixed with sand and placed in the painted terracotta pots on the surface of the board. Two odours (cinnamon 1% w/w, and cumin 0.4% w/w) and two locations (67 cm apart, 43.5 cm from the door of the start box, C and E in Figure 2.1, see p45) were used to create two correct and two incorrect pairings. The particular combination of odour and place deemed correct or incorrect remained the same for each rat throughout testing, but was counterbalanced across rats and within homecages. On each trial, one cup containing sand mixed with the powdered odour was placed in one of the locations on the board. It is important to note that only one pot was present on the board during each trial, but the location of this pot shifted between two positions (see Figure 2.1, p45). Rats learned to dig in the cup when a correct combination of odour and place was presented and to withhold digging if an incorrect combination was presented. A food reward (Kelloggs Froot Loop cereal) was buried in the sand on correct trials. Rats were presented with six correct and six incorrect trials per day, 5 days per week, over 14 weeks of testing. Rats began each trial in the start box. The time from when the rat's back feet exited the start box to when it began digging in the sand was recorded. Digging was defined as 2 or more

consecutive strokes in the sand with one or both front paws. Resting the front paws on the sand or making swiping motions that did not touch the sand were not counted. If a rat did not begin digging within 10 seconds of exiting the start box, a 10 second latency was recorded for that trial and the rat was returned to the start box for the next trial.

2.4.4 Data Analysis

Data analysis followed that described by Gilbert and Kesner (2002, 2003). For each rat, the average latency for rewarded trials and non-rewarded trials for each week of training was calculated. The average latency for rewarded trials was subtracted from the average latency for non-rewarded trials to give the average latency difference score for that week. Thus higher latency difference scores indicated that the time to digging was longer on non-rewarded trials than on rewarded trials.

2.5 Spatial probe trials

2.5.1 Spatial probe trial testing

Immediately following the 14 weeks on the standard odour-place paired-associate task, rats began the spatial probe task. It is important to note that there was no break between the end of the odour-place paired-associate task and the start of the spatial probe trials. The probe task was designed to examine the degree to which performance on the odour-place association task could be accounted for by response (egocentric) learning rather than spatial (allocentric) learning. Rats received 12 trials per day, 5 days per week. On half of these trials, the start box was in Location A (the original start location used in the odour-place association task), and on the other half it was in Location B (a new location directly opposite the original start location, see Figure 2.1, p45). The sequence of presentation of odours and pot locations was the same as in the standard odour-place paired-associate task for any given rat. For each rat, the location of the start box was randomly assigned and balanced so that there were approximately the same number of Location A and Location B trials for each odour-place combination each day and varied over the week. The procedure was otherwise the same as that described in the odour-place association task, in that rats received 12 trials per day, 5 days per week. Probe testing continued for three weeks.

2.5.2 Data analysis for spatial probe trials

Data were recorded and averaged in the same way as the odour-place association task. The only difference was that data for the original and new start positions were averaged separately, so the each rat had two weekly average latency difference scores: one for the original start box position and one for the new position.

2.6 Simple discrimination tasks

After completing the spontaneous object recognition task (see Section 2.7, p54), rats were tested for simple discrimination of either the spatial locations or the odours used in the odour-object association and spatial probe tasks. Testing began within a week after the spontaneous object recognition task. Half of the rats were assigned to a simple odour discrimination task, and half were assigned to a simple spatial discrimination task. Assignment to the two tasks was balanced across lesion groups, homecages and performance on the odour-place paired-associate task.

2.6.1 Simple odour discrimination task

The same two odours used in the odour-place association task (cinnamon, 1% w/w and cumin, 0.4% w/w) were used in this task. Powdered odours were mixed with sand and placed in a black painted pot in line with and 28 cm away from the start box (see Figure 2.1, p45). Rats learned to dig when the 'correct' odour was presented and to withhold digging if the 'incorrect' odour was presented. Designation of 'correct' and 'incorrect' odours was balanced across rats. The order of presentation of odours was determined by following pseudo-random sequences for choice tasks (Fellows, 1967). On each trial, the rat was placed in the start box at A (Figure 2.1, p45). The door was opened, and the rat was allowed to approach the cup and dig in the odourised sand. A Froot Loop reward was buried in the sand on correct trials. The latency from when the rat's back feet exited the start box to when the rat began digging in the pot was recorded. Digging was defined in the same way as in the odour-place paired-associate task.

2.6.2 Simple spatial discrimination task

The same two spatial locations used in the odour-place paired-associate task (see Figure 2.1, p45) were used in this task. Unscented sand was placed in a black painted pot in one of the two

locations used in the odour-place paired-associate task. Rats learned to dig when the pot was presented in the 'correct' location and to withhold digging if the pot was presented in the 'incorrect' location. Designation of 'correct' and 'incorrect' locations was balanced across rats. The order of locations was determined by following pseudo-random sequences for choice tasks (Fellows, 1967). Trials proceeded in the same way as described in the simple odour discrimination task. For both the simple odour discrimination and simple place discrimination tasks, rats received 12 trials per day, 5 days per week, until they reached a criterion of at least 10 correct trials on each of 2 consecutive days. A correct trial was defined as less than 2 seconds latency on a reward trial, or 10 seconds latency on a non-reward trial.

2.6.3 Data analysis for the simple discrimination tasks

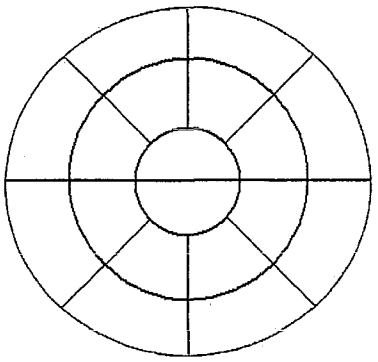
Latency data were recorded in the same way as the previous two tasks. Rats' responses were designated 'correct' if the latency was less than 2 seconds on a rewarded trial, or at least 10 seconds on a non-rewarded trial. The number of 'correct' responses (out of 12) for each rat for each day was calculated. Once a rat reached a criterion of at least 10 correct responses on each of two consecutive test days it was removed from the experiment and the number of days taken to reach criterion was recorded. Rats continued in the discrimination tasks until they reached criterion.

2.7 Spontaneous object recognition tasks

2.7.1 Spontaneous object recognition testing

Approximately one week after completing the odour-place paired-associate spatial probe task, rats performed the spontaneous object recognition tasks. The spontaneous object recognition testing consisted of seven consecutive six-minute long sessions, with a three minute break between sessions, all completed in a single day. The objects and the surface and walls of the apparatus were washed and dried before each session. Rats were placed alone in an empty cage in the experimental room for three minutes immediately prior to each session. In each session, rats were placed on the apparatus in the start position (see Figure 2.2, p47), facing the wall of the apparatus. They were then left alone in the experimental room for six minutes. Their activity was recorded via a small camera mounted on the ceiling of the room. Figure 2.4 (p56) shows the sequence of sessions for a single rat. In Session 1 the apparatus had no objects on it. In Sessions 2 to 4, five objects (the glass bottle,

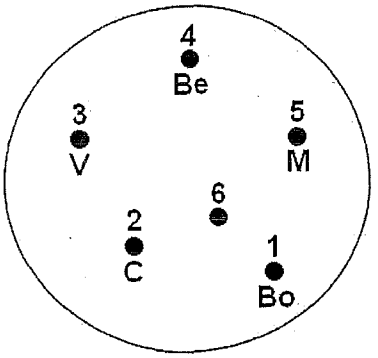
painted clay ornament, glass vase, plastic bed leg, and plastic painted monkey) were placed on the apparatus in locations 1 to 5 respectively (see Figure 2.2, p47). There was no object in location 6. In Sessions 5 and 6, the glass bottle moved to location 3 (new object-place combination using a familiar object moved to a familiar place) and the glass vase moved to location 6 (new object-place combination using a familiar object in a new place); there was no object in location 1. In Session 7, the painted clay ornament was removed and the plastic soap holder appeared in its place (new object).



Session 1

Blank board

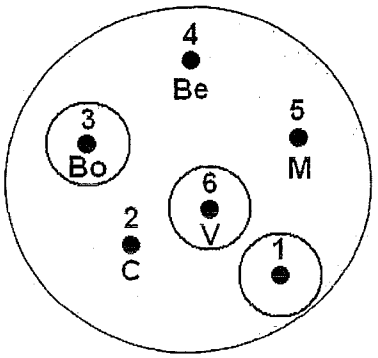
Locomotion data coding lines shown



Sessions 2 to 4

Locations of objects before the spatial change

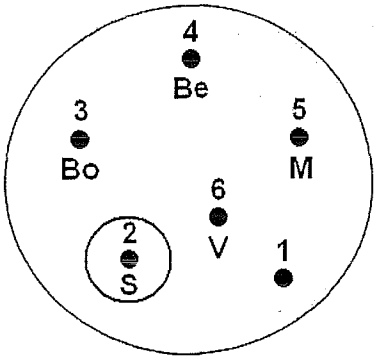
Note: Data coding lines not shown



Sessions 5 to 6

Locations of objects after the spatial change

Note: Data coding lines not shown



Session 7

Locations of objects after the object change

Note: Data coding lines not shown

Figure 2.4 Layout of the objects during the spontaneous object recognition task. Be= bedleg, M= monkey, Bo= bottle, C= painted ceramic ornament, V= vase, S= soap holder. Circled objects/locations indicate a change from the previous session.

2.7.2 Data analysis for spontaneous object recognition testing

Data from video recordings were analysed once all animals had completed the spontaneous object recognition task. Both movement around the apparatus and exploration of the objects were recorded. Locomotion was measured by counting the number of approximately equal sized areas that a rat entered (back feet) throughout each six-minute session. The areas were marked out on the apparatus before testing began and were clearly visible in the video recordings (see Figure 2.4, Session 1, p56). Exploration of objects was calculated by measuring the time spent exploring each object during each six-minute session. "Exploring" was defined as: the rat's nose being within 2 cm of an object and oriented towards it and actively sniffing it or touching it. Climbing onto an object or using it as support for rearing without otherwise exploring it was not counted as 'exploring'. The total time spent exploring each object was recorded. Acquisition of these records was conducted using an in-house computer program.

2.8 Histology

Following the conclusion of testing, animals were overdosed with sodium pentabarbitone and perfused transcardially with saline followed by 4% formalin solution. The brains were removed and stored in 4% formalin solution for 24 hours before being transferred to long-term sucrose storage solution. Brains were frozen and cut on a cryostat at 50 μm. Every section throughout the thalamus was taken and mounted onto slides. Sections were stained with cresyl violet and lesion placements were verified by microscope examination. Satisfactory lesions met the criteria of at least 50% damage to the intended thalamic aggregate and less than 40% damage to either of the other two thalamic aggregates. Only rats with lesions that met these criteria were included in the behavioural analyses.

2.9 Power and sample size considerations

When determining the sample size for the experiment, several considerations were made. Firstly, the sample size must be large enough to reliably detect any group differences in performance on the task. Secondly, the sample size must be practical to enable all animals to be tested on a single day. Finally, the sample size must be large enough to allow some animals to be excluded in case of inaccurate lesion placements. As no published studies have directly compared performance between

three selective thalamic lesion groups on an odour-place paired-associate task, the effect size was estimated from other studies that used fewer lesion groups and from unpublished work on these lesions at the University of Canterbury. Studies comparing performance of AT lesion animals to controls on tasks involving a spatial component have typically yielded very large effect sizes (for example see Mitchell & Dalrymple-Alford, 2005; Sziklas & Petrides, 1999). Previous work from the University of Canterbury (Mitchell & Dalrymple-Alford 2005) has suggested that there will likely be no difference in mean performance on the odour-place paired-associate task between control and MT animals, a mild difference, perhaps d=0.50, between control and LT animals, and a large difference, perhaps d=1.50, between AT and control animals. Based on these previous findings, an estimated effect size for a MANOVA design comparing the three lesion groups plus a control group was f=0.61. With this effect size, eight rats per group would give 78% power. A further consideration was the accuracy of lesion placements. Because of the highly selective nature of the lesions and the criteria used to evaluate lesion placements, it was likely that a few animals might be excluded because of inaccurate lesion placements. Given the estimated effect size, the possibility of some animals being excluded from analysis, and the constraint that all animals must be tested in a single day, the final sample size used was 36 animals (9 animals per lesion group).

One animal died under anaesthetic, leaving a total sample size of 35 animals. Of these, two MT animals had lesions that did not fulfil our criteria and were excluded from behavioural analysis, leaving final group sizes of AT=9, MT=7, LT=8 and Control=9.

3. Results

3.1 Histological findings

Acceptable lesions were defined as having more than 50% bilateral damage to the intended aggregate and not more than 40% bilateral damage to either of the other two thalamic aggregates. Table 3.1 (pp63-64) shows the extent of damage to thalamic structures for included and any excluded cases (only two MT rats) for each of the three lesion groups. The two excluded MT lesions had insufficient damage to the intended aggregate and were excluded from the behavioural analyses. Figures 3.1, 3.2 and 3.3 (pp60-62) show the largest and smallest included lesions (by % damage to the intended aggregate) in each of the three lesion groups.

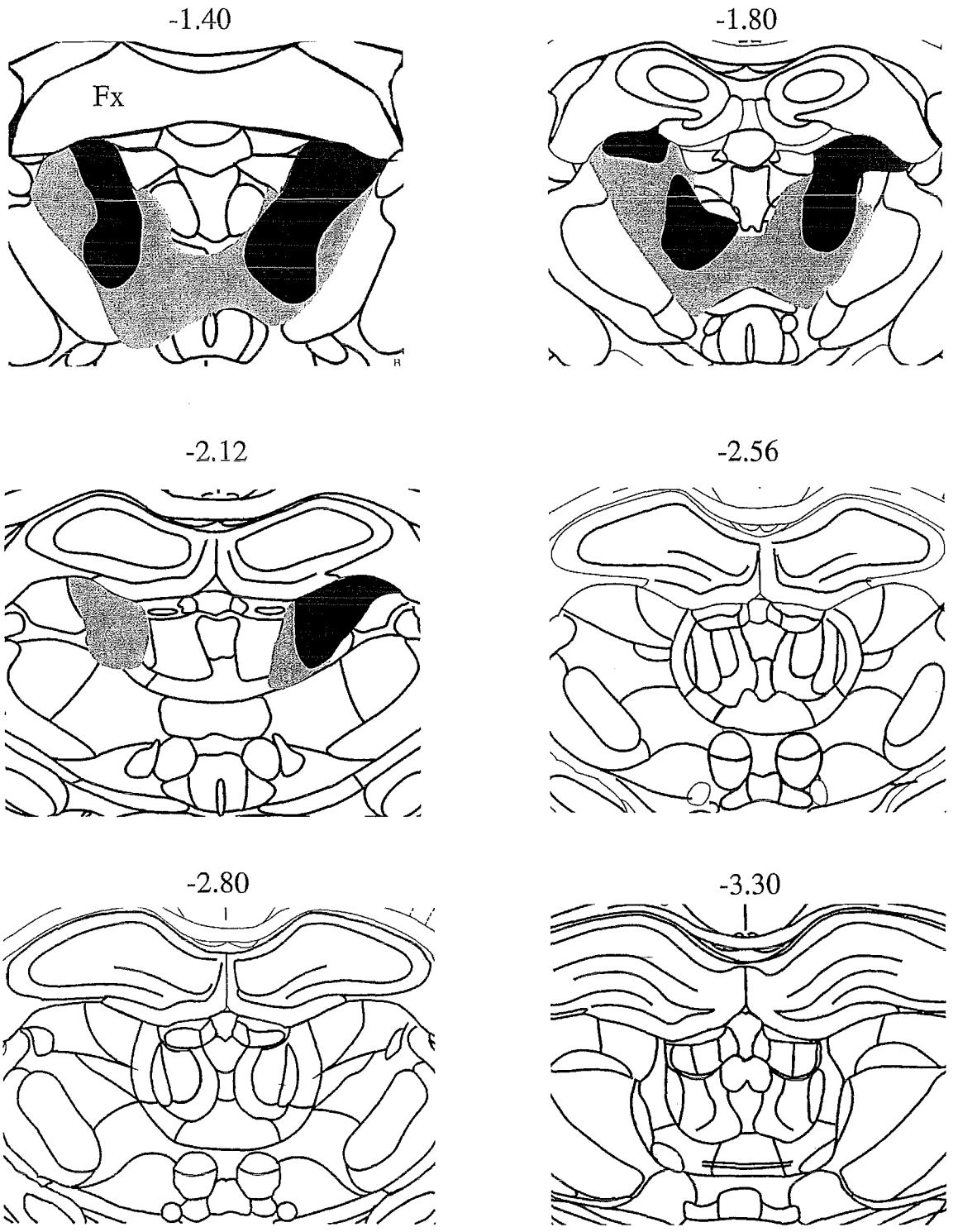


Figure 3.1 Schematic coronal sections through the rat brain (Paxinos & Watson, 1998) showing the largest (grey) and smallest (black) included lesions in the AT group. Numbers are distances from Bregma. Fx= fimbria-fornix.

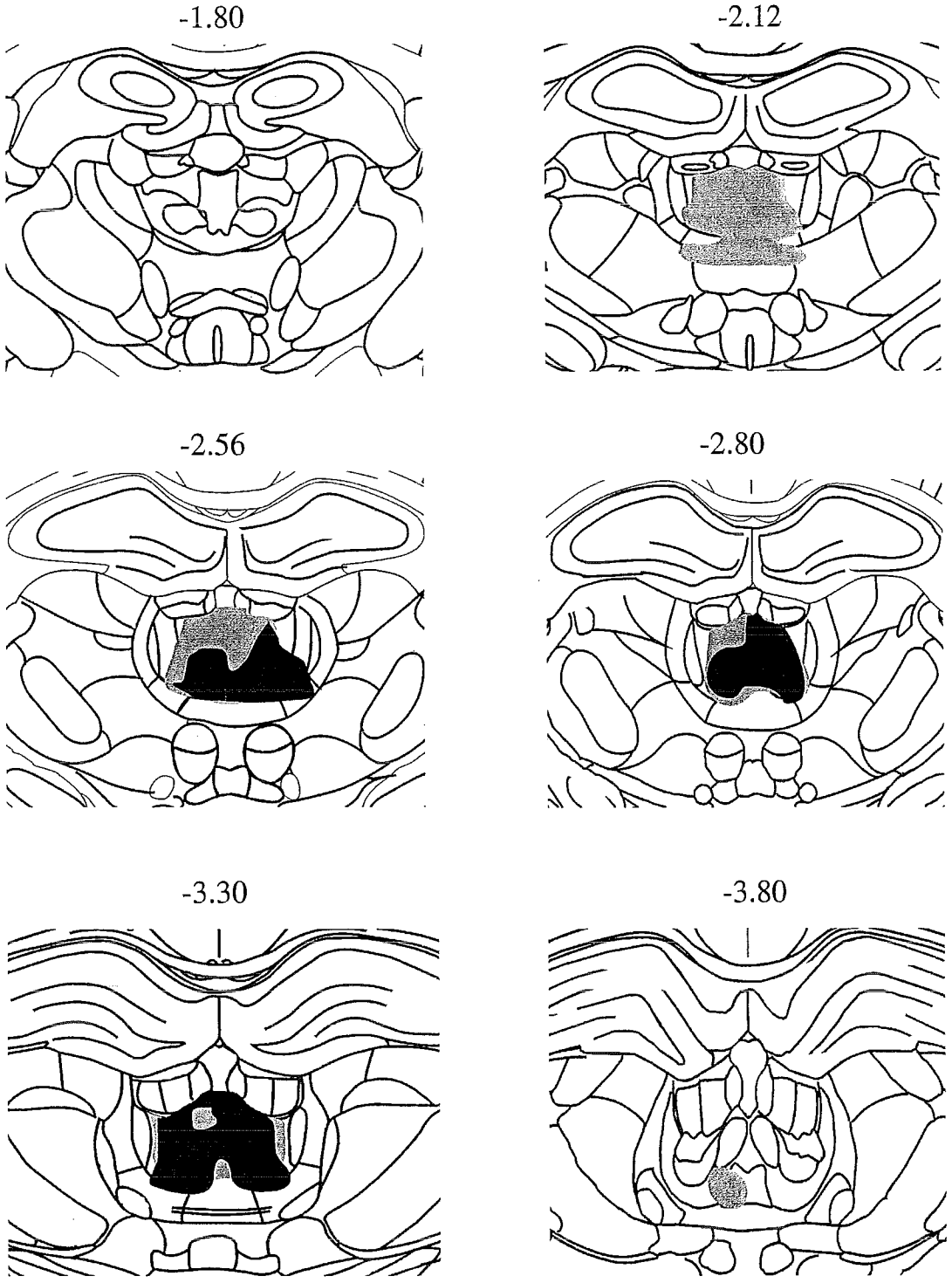


Figure 3.2. Schematic coronal sections through the rat brain (Paxinos & Watson, 1998) showing the locations of the largest (grey) and smallest (black) included lesions in the MT group. Numbers are distances from Bregma.

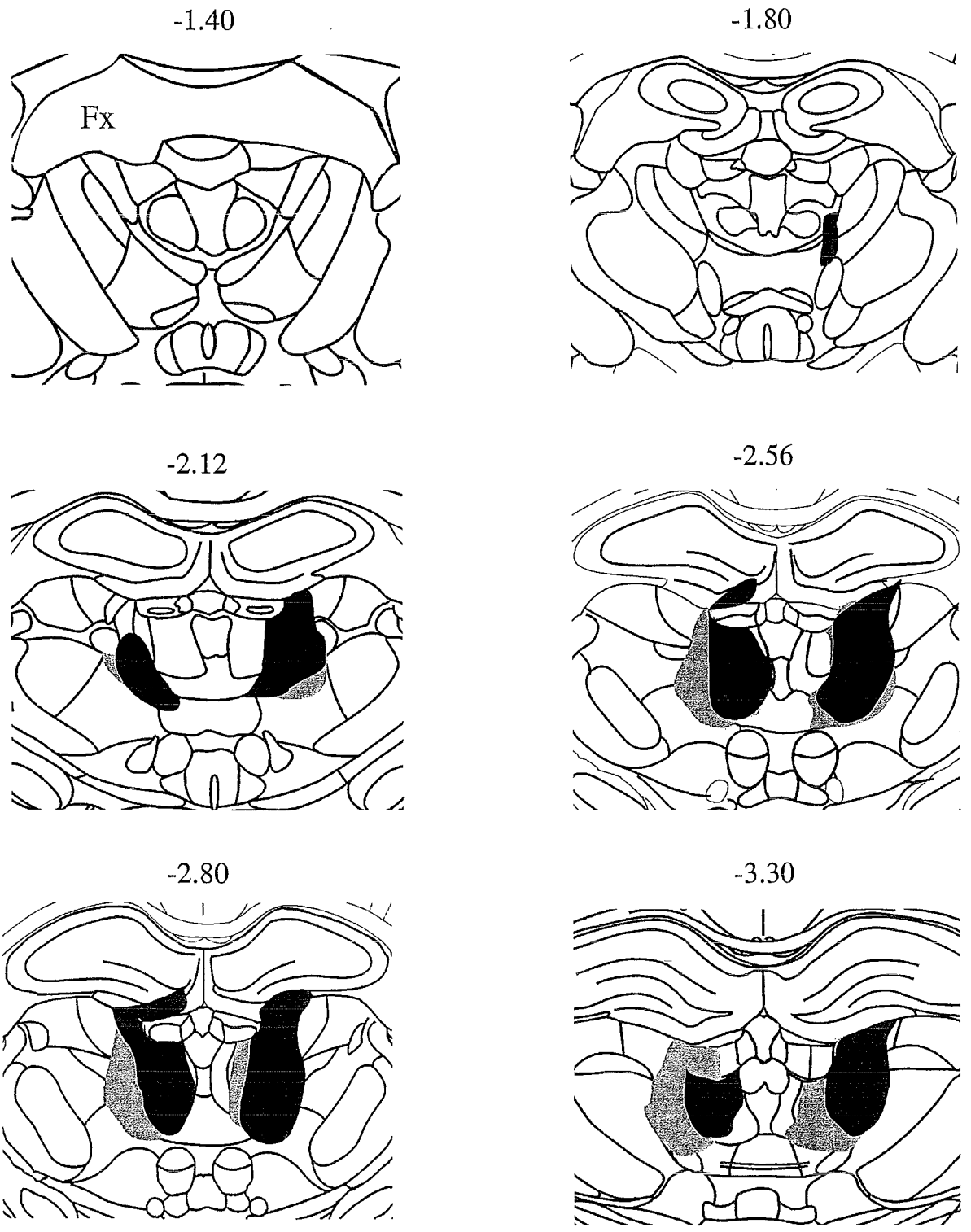


Figure 3.3 Schematic coronal sections through the rat brain (Paxinos & Watson, 1998) showing the locations of the largest (grey) and smallest (black) included lesions in the LT group. Numbers are distances from Bregma.

Table 3.1 Percent bilateral damage (volume) to selected areas for each of the rats in the study.

	AT and components				MT and components					LT and components						Other nuclei						
	AD	AM	AV	AT	IMD	MDc	MDm	MD	MT	CL	MDl	MDpl	PC	CMr	LT	IAM	LD	PT	PVA	PV/ PVP	Re	Rh
AT Inclusions																						
6R	76.1	38.0	71.7	54.2	0.0	1.4	0.0	25.5	2.1	4.4	1.6	0.0	13.2	6.9	6.1	7.2	6.1	17.7	0.1	0.0	0.0	0.0
3G	86.7	29.9	70.8	56.0	0.0	0.0	0.0	16.8	1.1	3.2	0.0	0.0	9.6	1.1	3.6	11.6	4.5	7.8	0.0	0.0	0.0	0.0
9G	60.5	65.3	69.8	64.6	0.0	0.1	2.7	39.8	4.1	4.7	4.5	0.0	18.3	16.2	9.5	34.7	8.9	19.4	0.0	0.0	0.0	0.0
5R	66.0	72.8	61.5	66.1	0.0	0.0	7.4	70.5	8.8	6.3	1.5	0.0	22.4	21.2	10.9	57.6	13.7	63.6	46.5	0.0	0.8	8.8
3P	98.9	44.6	81.2	69.7	0.0	0.0	0.0	30.6	2.0	9.3	0.8	0.0	12.4	1.3	6.7	15.0	6.0	6.3	0.0	0.0	2.6	7.1
1R	89.9	43.9	78.0	74.0	0.0	0.0	0.0	27.0	1.7	6.9	1.8	0.0	18.1	5.2	8.0	7.1	5.0	10.6	0.0	0.0	0.4	0.0
4B	99.8	63.4	64.6	74.9	0.0	0.0	0.0	27.3	1.7	5.0	2.1	0.0	13.7	1.7	5.9	18.2	2.9	8.2	0.0	0.0	0.0	0.1
4G	97.1	80.5	100.0	84.5	0.0	18.6	8.9	67.1	14.7	38.4	31.6	94.0	33.3	36.2	35.7	66.7	18.3	39.0	0.9	0.0	0.0	4.8
1B	99.2	90.1	71.0	91.0	0.0	0.1	0.4	48.3	3.3	9.2	7.5	0.0	20.5	15.1	12.2	56.9	7.3	21.6	0.1	0.0	3.5	3.6
AT median N=9	89.9	63.4	71.0	69.7	0.0	0.0	0.0	30.6	2.1	6.3	1.8	0.0	18.1	6.9	8.0	18.2	6.1	17.7	0.0	0.0	0.0	0.1
LT Inclusions																						
10R	3.6	6.2	17.6	4.3	0.7	61.9	26.1	0.0	32.7	56.0	75.1	65.5	32.2	17.6	50.1	3.4	8.4	0.0	0.0	0.6	0.0	0.0
6B	15.1	12.7	25.9	13.3	0.0	69.0	17.8	8.5	30.4	58.0	70.9	100.0	41.1	25.1	53.3	4.2	3.3	1.3	0.0	0.0	0.0	0.0
9P	0.3	2.9	10.7	2.8	0.0	37.4	10.0	0.4	16.4	78.0	61.5	100.0	39.1	16.4	56.1	0.0	1.8	0.2	0.0	0.0	0.0	0.0
8R	0.0	0.0	0.0	0.0	0.0	63.8	21.1	0.0	30.3	70.8	77.8	88.5	42.9	3.3	56.9	0.0	1.4	0.0	0.0	1.2	0.0	0.0
7G	3.0	10.1	11.9	5.7	0.0	74.2	26.2	0.3	36.2	72.3	80.5	100.0	52.4	32.0	64.5	2.6	2.7	0.0	0.0	0.0	0.0	0.5
7B	3.6	10.2	39.4	9.0	0.2	72.6	30.0	0.0	37.9	81.9	87.0	100.0	43.6	22.8	66.1	0.9	3.1	0.0	0.0	0.0	0.0	0.0
9B	7.1	0.2	37.2	2.9	0.0	73.0	12.0	0.0	27.7	90.3	87.8	100.0	41.4	6.9	66.6	0.0	6.2	0.0	0.0	0.0	0.0	0.0
7R	0.0	0.8	13.5	0.9	0.0	67.8	22.0	0.0	32.0	89.0	87.0	100.0	55.4	16.3	70.6	0.7	2.0	0.0	0.0	0.0	0.0	0.2
LT Median N=8	3.0	2.9	13.5	2.9	0.0	69.0	21.1	0.0	30.4	78.0	80.5	100.0	42.9	16.4	64.5	0.7	2.7	0.0	0.0	0.0	0.0	0.0

Table 3.1 continued

	AT and components				MT and components					LT and components						Other nuclei						
	AD	AM	AV	AT	IMD	MDc	MDm	MD	MT	CL	MDl	MDpl	PC	CMr	LT	IAM	LD	PT	PVA	PV/ PVP	Re	Rh
MT Inclusions																						
5B	0.0	0.0	0.0	0.0	70.1	54.7	56.1	0.0	53.1	0.0	5.8	0.0	6.6	11.3	4.7	0.0	0.0	0.0	0.0	30.6	0.0	0.0
6G	0.0	0.0	0.0	0.0	87.1	45.8	64.9	0.0	57.0	0.0	1.5	0.0	0.1	2.1	0.7	0.0	0.0	0.0	0.0	62.0	0.0	0.0
3B	0.0	0.0	0.0	0.0	88.7	48.4	64.9	0.0	57.8	0.0	5.4	0.0	0.9	10.1	3.0	0.0	0.0	0.0	0.1	52.0	0.0	0.0
2P	0.0	0.0	0.0	0.0	64.4	47.0	66.6	0.0	66.6	0.2	12.7	0.0	16.2	20.3	10.2	7.5	0.0	0.0	0.0	53.7	0.0	3.9
4P	0.0	0.0	0.0	0.0	81.5	72.1	68.7	44.9	69.1	0.0	1.5	0.0	2.3	23.0	4.0	0.0	0.0	9.7	18.7	51.8	0.0	0.0
9R	0.0	0.0	0.0	0.0	100.0	74.9	74.3	0.0	71.6	0.0	8.0	0.0	3.2	17.5	5.2	0.0	0.0	0.0	1.4	72.0	0.0	0.0
3R	0.0	1.7	0.0	0.5	95.9	70.4	78.6	0.6	72.6	0.0	10.4	0.0	2.2	42.0	8.9	4.0	0.0	2.7	10.9	67.6	0.0	0.0
MT median N=7	0.0	0.0	0.0	0.0	87.1	54.7	66.6	0.0	66.6	0.0	5.8	0.0	2.3	17.5	4.7	0.0	0.0	0.0	0.1	53.7	0.0	0.0
MT Exclusions																						
2R	0.0	0.0	0.0	0.0	32.8	0.0	3.3	0.0	4.3	0.0	0.0	0.0	0.0	7.7	1.0	0.0	0.0	0.0	10.7	33.5	0.0	0.0
5G	0.0	0.0	0.0	0.0	39.6	0.0	3.2	0.0	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	46.8	0.0	0.0

Abbreviations: AD= anterodorsal nucleus; AM= anteromedial nucleus; AT= anterior thalamic aggregate; AT median= median percent damage for all included AT rats; AV= anteroventral nucleus; CL= centrolateral nuclei; CMr= rostral central medial nuclei; IAM= interanterodorsal nucleus; IMD= intermediodorsal nucleus; LD= laterodorsal nucleus; LT= lateral thalamic aggregate; LT median= median percent damage for all included LT rats; MD= mediodorsal nucleus; MDc= central segment of the mediodorsal nucleus; MDl= lateral segment of the mediodorsal nucleus; MDm= medial segment of the mediodorsal nucleus; MDpl= paralamellar segment of the mediodorsal nucleus; MT= posteromedial thalamic aggregate; MT median= median percent damage for all included MT rats; PC= paracentral nucleus; PT= paratenial nucleus; PVA= anterior paraventricular nucleus; PV/PVP= paraventricular nucleus/posterior paraventricular nucleus; Re= reunions nucleus; Rh= rhomboid nucleus.

3.2 Odour-place paired-associate task

The go/no-go odour-place paired-associate task required animals to form arbitrary associations between odours and places. The behavioural data analysis for this task followed that used by Gilbert & Kesner (2002; 2003). The dependent measure was the latency from hind feet exiting the start box to digging in the sand pot. For each test day, the average latency for rewarded trials was subtracted from the average latency for non-rewarded trials to produce an average latency difference score. Optimal performance on the odour-place paired-associate task required that rats withhold responses for the 10 second maximum time on non-rewarded trials, but respond quickly on rewarded trials. High average latency difference scores indicate that rats were withholding responses on non-rewarded trials but responding quickly on rewarded trials.

Observations of the rats' behaviour revealed that after exiting the start box they always travelled in a straight line directly towards the single digging cup present on the board, irrespective of position. In the first weeks of the task the rats had very short latencies on both rewarded and non-rewarded trials, but over time some rats gradually learned to withhold responses on non-rewarded trials.

Figure 3.4 (p67) shows the average latency difference scores for the four groups over the 14 weeks of testing. The figure shows that the MT and Control groups gradually acquired the association between the odours and spatial locations over the 14 weeks of testing, beginning on average after about 5 to 6 weeks of training. There was little difference in scores between the MT and Control groups. The average latency difference scores for the LT group increased only slowly towards the latter half of the 14 week testing period. The AT group showed almost no increase in latency difference scores over the entire 14 weeks of testing. Hence both LT and AT groups were impaired at learning the association relative to the MT and Control groups.

A 4(Lesion) × 14(Week) repeated measures MANOVA (Statistica) confirmed these observations. There were highly significant effects for Lesion ($F(3,29)=6.49$, $p<0.002$), Week ($F(13,377)=52.06$, $p<0.0001$) and Lesion × Week interaction ($F(39,377)=4.83$, $p<0.0001$). Post-hoc comparisons (Newman Keuls) confirmed that the both the AT group ($p<0.02$) and LT groups ($p<0.04$) were significantly impaired relative to the Control group; they were also significantly impaired relative to the MT group (AT: $p<0.004$; LT: $p<0.02$). There was no significant difference between the MT and Control groups ($p>0.44$). Although the graph suggests that the LT group performed slightly better than the AT group, there was no significant difference between these

groups ($p>0.43$). Figure 3.4 (p76) shows that during the last 4 weeks of training especially the performance of the LT group appears to diverge from the performance of the AT group, which remained poor. A 4(Lesion) × 4(Latter 4 weeks) MANOVA using the last 4 weeks of data revealed a significant effect for Lesion ($F(3,29)=8.02$, $p<0.001$). Post-hoc comparisons (Newman Keuls) revealed significant differences between the AT group and the Control ($p<0.01$) and MT ($p<0.01$) groups, and between the LT group and the Control ($p<0.03$) and MT ($p<0.02$) groups, but not between the MT and Control groups ($p>0.52$). Despite the apparent divergence of the AT and LT groups on the final four weeks of the task, there was no significant difference between these two groups ($p>0.27$).

Figure 3.5 (p68) shows the average increase in latency difference scores between the start and end of training (weeks 1 and 14). These difference scores were examined to determine the amount of learning that occurred over the entire training period. The figure shows that, while the average latency difference increased considerably for the MT and Control groups, it increased only a small amount for the LT group, and hardly any for the AT group. A one-way ANOVA revealed a significant difference between the four lesion groups ($F(3,29)=9.15$, $p<0.001$). Post-hoc comparisons (Newman Keuls) revealed that the AT group showed a significantly smaller increase in latency difference scores than the Control ($p<0.01$) and MT ($p<0.01$) groups. The LT group also showed a significantly smaller increase in latency difference scores than the Control ($p<0.05$) and MT ($p<0.01$) groups. There was no significant difference between the AT and LT groups ($p>0.28$) or between the MT and Control groups ($p>0.93$).

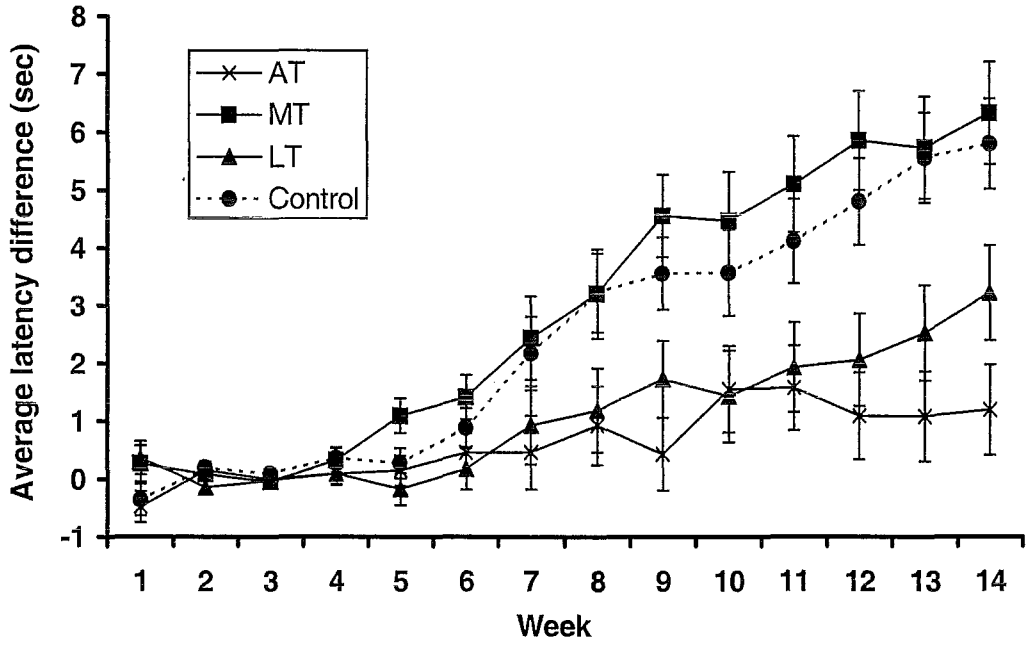


Figure 3.4 Average latency differences (sec) on the odour-place paired-associate task for the AT, MT, LT and Control groups over the 14-week testing period. Vertical bars are ± standard error of the mean (SEM).

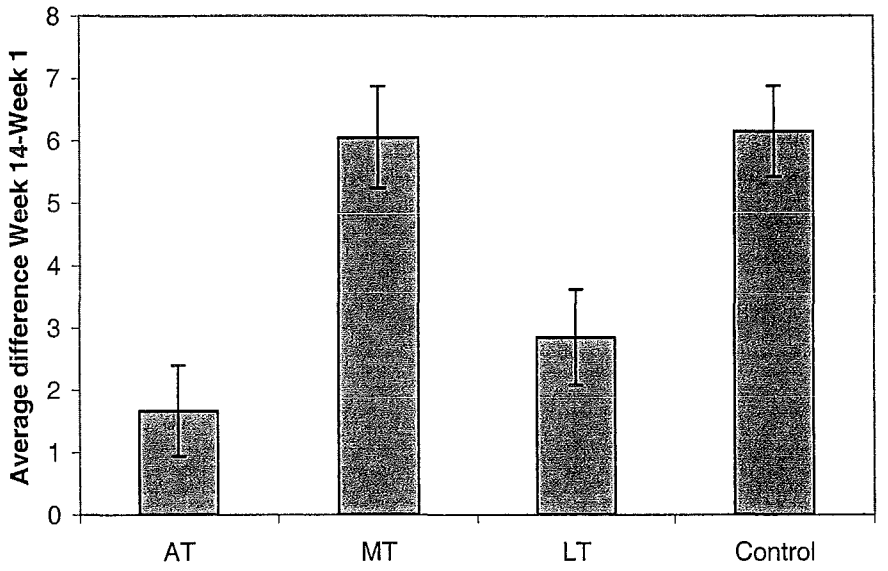


Figure 3.5 Average change in latency difference scores between Week 1 and Week 14 of the odour-place paired-associate task. Vertical lines are ±SEM.

To explore further differences between groups, and to compare with the subsequent simple discrimination tasks (see Section 3.4, p73), the average number of days to criterion on the odour-place paired-associate task was also calculated. The criterion used was at least 10 correct responses on each of two consecutive test days. 'Correct' responses were latencies of less than 2 seconds on rewarded trials or 10 seconds on non-rewarded trials. If a rat had not reached criterion by the end of the 14-week testing period, their score was recorded as the number of days completed (70 days) plus an extra week (5 days). Figure 3.6 (p69) shows the average number of days to criterion on the odour-place paired-associate task for each of the AT, MT and LT groups. The number of rats that reached criterion in each group was: AT 2/9; MT 5/7; LT 3/8; Control 7/9. As expected from Figure 3.4 (p76), the AT and LT groups required more days to reach criterion than the Control and MT groups (Figure 3.6, p69). There appeared to be little difference between the MT and Control groups or between the AT and LT groups. A one-way ANOVA confirmed a significant difference between the four groups in the number of days to criterion ($F(3,29)=3.67$, $p<0.03$). Post-hoc tests (Newman Keuls) revealed that the number of days for the AT group was significantly higher than the number for the MT group ($p<0.05$). Despite the apparent differences in the graph there were no other significant differences between the groups, although the comparison between the AT and Control

groups just failed to reach significance ($p<0.06$). There was no significant difference between the AT and LT groups ($p>0.50$), MT and Control groups ($p>0.74$), but the comparisons between MT and LT groups ($p>0.10$) and LT and Control groups ($p>0.08$) also approached significance.

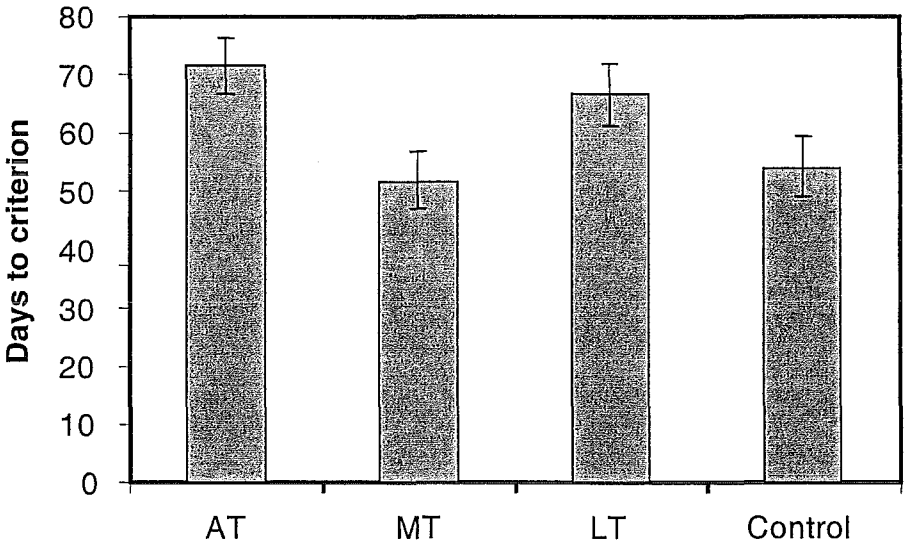


Figure 3.6 Average number of days to criterion on the odour-place paired-associate task for the AT, MT, LT and Control groups. Vertical bars are ±SEM.

3.3 Spatial probe tasks

The aim of the spatial probe task was to examine whether rats were relying on allocentric or egocentric cues, or a combination of both, to solve the odour-place paired-associate task. The dependent measure was the same as for the odour-place association task (average latency difference score). Again, optimal performance on spatial probe task required that rats withhold responses for the maximum 10-seconds on non-rewarded trials, but respond considerably faster on rewarded trials. High average latency difference scores indicate that rats were withholding responses on non-rewarded trials but responding quickly on rewarded trials. Average latency differences from the spatial probe task were compared to the last 2 weeks of the odour-place paired-associate task to assess which "strategies" animals were using. A drop in average latency difference scores on trials

where the start box is in the new position would suggest that rats may be relying partially or wholly on egocentric cues or some other cue or strategy. A recovery of average latency difference scores over the three weeks of testing on the spatial probe task would indicate that rats had adjusted their strategy to rely more on allocentric cues.

Observations of the rats' behaviour on new start position trials revealed that rats continued to exit the start box and travel in a straight line towards the digging cup. They did not travel to the old start box position before approaching the cup.

Figure 3.7 (p72) shows the average latency difference scores for the three weeks of the spatial probe task relative to the last two weeks of acquisition (Week 13 and Week 14), and Table 3.2 shows the drop in performance in Week 1 of probe testing relative to Week 14 of acquisition. Separate scores are provided for trials where the start box was in the old position and the new position. The final two weeks of the odour-place paired-associate task are also provided for comparison. The probe trials revealed several interesting findings. Firstly, the introduction of the probe trials resulted in a drop in performance compared to the final weeks of the odour-place paired-associate task. Table 3.2 (p73) shows that for all groups, performance was lower in the first week of the probe trials than in the final week of the odour-place paired-associate task. The drop was greatest in the MT and Control groups, moderate in the LT group, and smallest in the AT group. In support of this, a 4(Lesion) × 2(Start position) MANOVA revealed a significant effect for Lesion ($F(3,29)=4.71$, $p<0.01$). There was a drop in performance on both the new and old start position trials, although for most groups the drop was greater on new start position trials. For the AT group this pattern was reversed, with a slightly greater drop on the old start position trials than the new start position trials. The MANOVA confirmed these observations, revealing a significant effect for Start position ($F(1,29)=12.79$, $p<0.01$) and a significant Lesion × Start position interaction ($F(3,29)=4.02$, $p<0.02$).

Figure 3.7 (p72) shows that in Week 1 especially performance was poorer on the new start position trials than the old start position trials for all groups except AT. A 4(Lesion) × 3(Week) × 2(Start position) MANOVA confirmed this, showing a significant effect for Start position ($F(1,29)=5.10$, $p<0.04$). Performance improved over the three weeks of the probe task for all groups except AT, and in support of this the MANOVA revealed a significant effect for Week ($F(2,58)=24.22$, $p<0.0001$). As in the odour-place paired-associate task, the MT and Control groups performed better overall than the LT and AT groups, with the LT group also performing slightly

better than the AT group, and the MANOVA revealed a main effect for Lesion ($F(3,29)=4.14$, $p<0.02$).

It is also important to note that the pattern of performance over the three weeks of probe testing was not the same for all four groups. The AT group, who performed poorly on the odour-place paired-associate task, continued to perform poorly on both the old and new start position trials during the probe task. Analysis of individual data for the two rats in the AT group that had showed some evidence of learning during the odour-place paired-associate task revealed that these animals showed a drop in performance after the introduction of the probe trials, but showed some recovery in performance over the three weeks of probe testing (see Appendix B for data for these two animals). The LT group was initially more impaired on the new start position trials, but performance on the new start position trials improved, and during weeks 2 and 3 there were no clear differences between performance on the new and old start positions. The MT and Control groups were more impaired on the new start position trials during the first week, but by weeks two and three there was little difference between performance on the new and old start positions. Another interesting finding was that, by week three, the MT group had reached a level of performance that was similar to their level of performance on the odour-place paired-associate task, but the Control group had not. At Week 3 the Control group showed performance that was considerably poorer than their performance on the final weeks of the odour-place paired-associate task. In support of these trends, the MANOVA revealed significant interactions for Week ×Start position ($F(2,58)=6.40$, $p<0.004$), Start position ×Lesion ($F(3,29)=4.78$, $p<0.008$) and Week × Lesion ($F(6.58)=4.49$, $p<0.001$). The Lesion × Week × Start position interaction was not significant ($F(6,58)=1.20$, $p>0.31$).

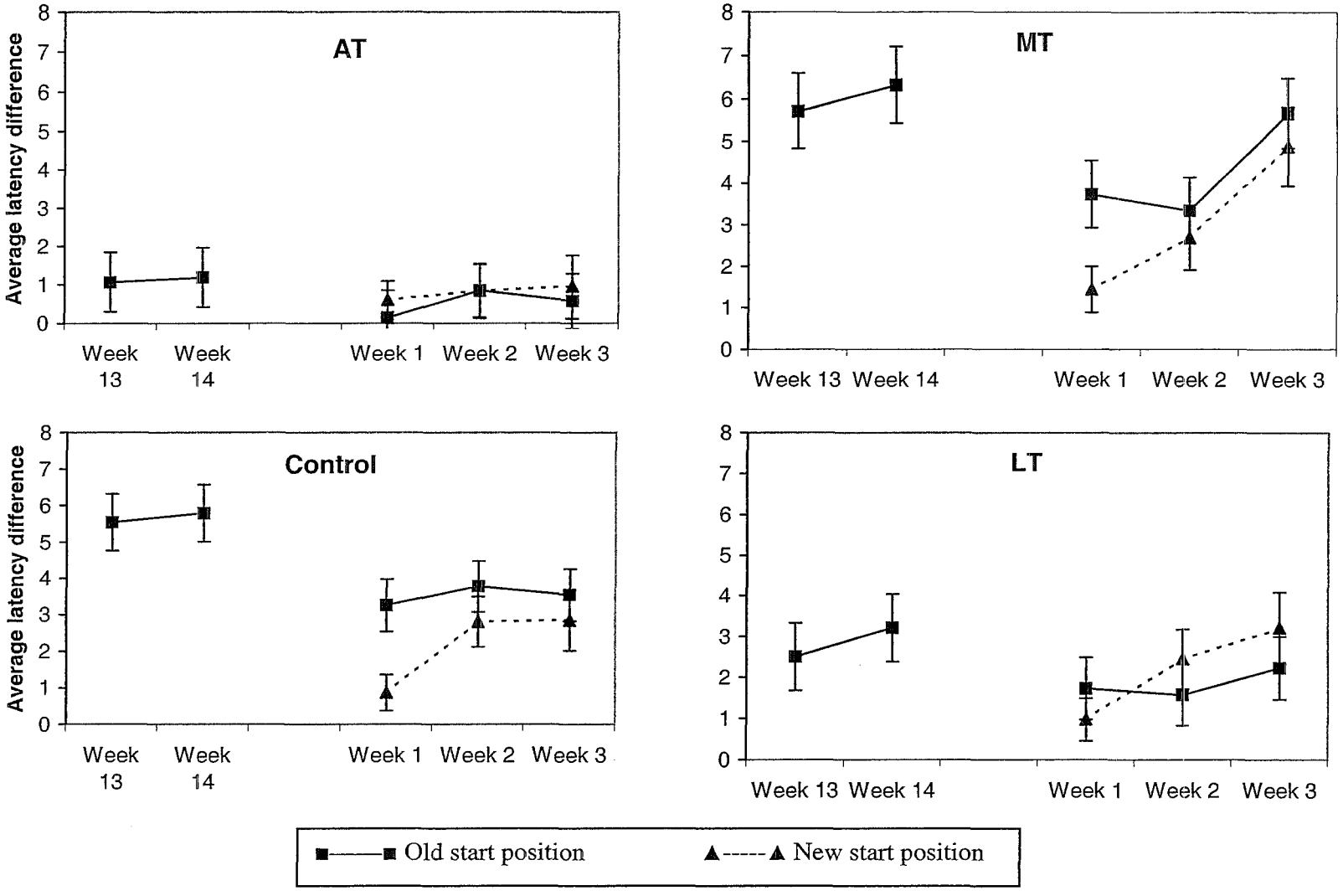


Figure 3.7 Average latency difference scores for the AT, MT, LT and Control groups for the final two weeks of the odour-place paired-associate task and the three weeks of the spatial probe task. Data for both 'old' and 'new' start positions are shown. Vertical bars are ± SEM

Table 3.2 Average drop in performance following introduction of the probe trials (Week 14 odour-place paired-associate task – Week 1 probe trials) for the AT, MT, LT and Control groups. Data are means (SEM).

	Drop in performance, Week 14 - Week 1 (drop in latency difference, seconds)		
	Old	New	Average
AT	1.05 (0.71)	0.59 (0.74)	0.82
MT	2.57 (0.80)	4.88 (0.84)	3.73
LT	1.47 (0.75)	2.22 (0.79)	1.85
Control	2.54 (0.71)	4.92 (0.74)	3.73
All groups	1.91	3.15	2.53

3.4 Simple discrimination tasks

Simple discrimination tasks were performed at the conclusion of testing to check that any impairments on the odour-place paired-associate task were not due to an inability to distinguish between the odours or places used in the task, or an inability to withhold digging responses. Half of the rats completed the odour discrimination task, and half completed the place discrimination task. The dependent measure on each task was the number of days required to reach a criterion of at least 10 correct responses on each of two consecutive test days. Correct responses were defined in the same way as previous for the odour-place paired-associate task.

Figure 3.8A (p75) shows the average number of days required to reach criterion on the spatial discrimination task for the four groups. Figure 3.8A shows that the MT and Control groups required a similar number of days to reach criterion. The LT and AT groups required more days to reach criterion than the MT and Control groups, with the AT group needing more days than the LT group. Despite these apparent trends, a one-way ANOVA showed no significant difference between the four groups in the number of days required to reach criterion ($F(3,11)=2.47$, $p>0.11$). Perhaps larger sample sizes might reveal slower acquisition of the task after AT lesions.

Figure 3.8B (p75) shows the average number of days required to reach criterion on the odour discrimination task for the four groups. Figure 3.8B shows that the MT, LT and Control groups all

required a similar number of days to reach criterion. The AT group had a slightly higher average number of days to criterion than the other three groups. A one-way ANOVA confirmed these observations, revealing a significant effect for Lesion ($F(3,14)=4.87$, $p<0.02$). Post-hoc comparisons indicated that the number of days to criterion was significantly greater for the AT group than for the MT ($p<0.04$), LT ($p<0.05$) and Control ($p<0.02$) groups. There were no other significant group differences.

One important point to note is that the number of days to criterion was far fewer for the simple discrimination tasks than for the odour-place paired-associate task. It seems unlikely that adaptation to general procedures (the odour-place paired-associate task was done first only) explains the extent of this difference. For example, while the subgroup of AT rats that completed the odour discrimination task was impaired relative to the LT, MT and Control groups, the performance of this AT subgroup was nevertheless considerably better than their performance on the odour-place paired-associate task. While all 5 rats in this AT subgroup completed the simple odour discrimination task to criterion within 11 days (with an average of 7.2 days), only 2 AT animals out of the entire AT group reached criterion after 70 days of training in the odour-place paired-associate task and one of these latter two AT rats completed the odour discrimination while the other completed the place discrimination (see Table 3.4, p99).

Figures 3.8A and 3.8B also show that, for all four groups, the number of days to criterion was higher on the spatial discrimination task than on the odour discrimination task. The difference appears greater in the AT and LT groups than in the MT and Control groups. To test these trends, an additional 4(Lesion) × 2(Task) ANOVA was conducted. The ANOVA revealed a significant main effect for Task ($F(1,25)=10.40$, $p<0.01$) and a significant main effect for Lesion ($F(3,25)=5.33$, $p<0.01$). The interaction effect was not significant ($F(3,25)=1.11$, $p>0.36$).

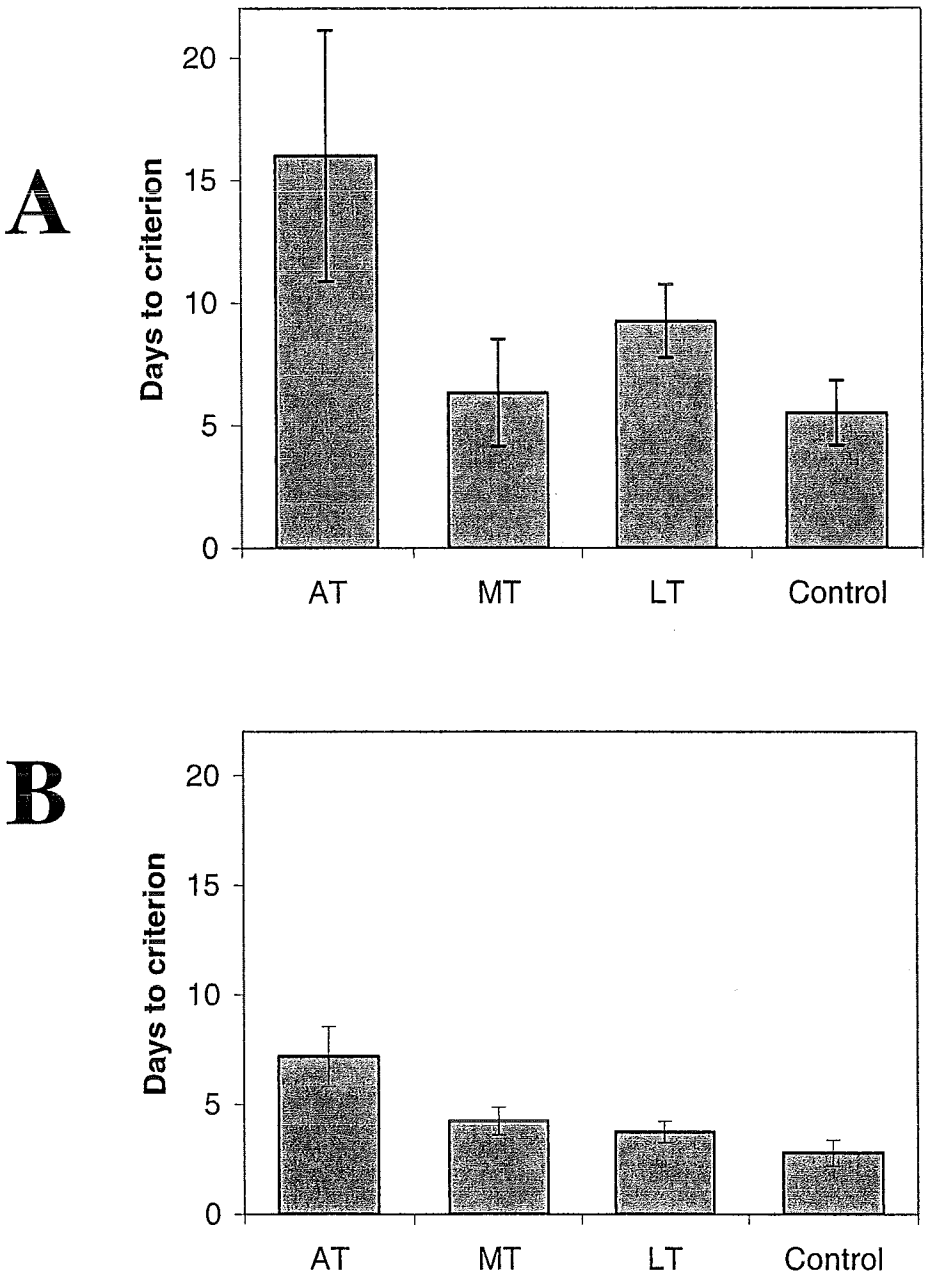


Figure 3.8 Average number of days required to reach criterion for the AT, MT, LT and Control groups on the place (A) and odour (B) discrimination tasks. Vertical bars are ±SEM.

3.5 Spontaneous object recognition task

The spontaneous object recognition task examined rats' responses to changes in objects and object-place associations. The dependent measures were the number of areas entered (locomotion) in each of seven six-minute sessions on the board and the time spent by rats exploring each of 5 objects, some novel and some familiar, in the last six sessions. The data analysis for the spontaneous object recognition tasks follows that used by Poucet (Save et al 1992).

3.5.1 Locomotion

Locomotion was measured as the number of marked areas of approximately equal size that were entered during each session. Figure 3.9 (p77) shows the number of areas entered during each six-minute session for the AT, MT, LT and Control groups. The number of areas entered decreased from Session 1 to Session 7 for all four groups. Although the overall rate of locomotion appeared similar for the four groups, the rate of decrease across Sessions varied slightly between the groups. Locomotion tended to be higher in AT and MT rats than LT and Control rats in the first two Sessions, similar in all groups in Sessions 3 and 4, but between Sessions 4 and 5 the AT, MT and LT groups showed a drop in locomotion, whereas the Control group did not. For Sessions 6 and 7 all groups showed a similar level of locomotion. A 4(Lesion) × 7(Session) MANOVA confirmed these trends, and revealed a significant main effect for Session ($F(6,174)=86.79$, $p<0.0001$), no Lesion effect ($F(3,29)=0.51$, $p>0.67$), but a significant interaction effect ($F(18,174)=2.08$, $p<.01$).

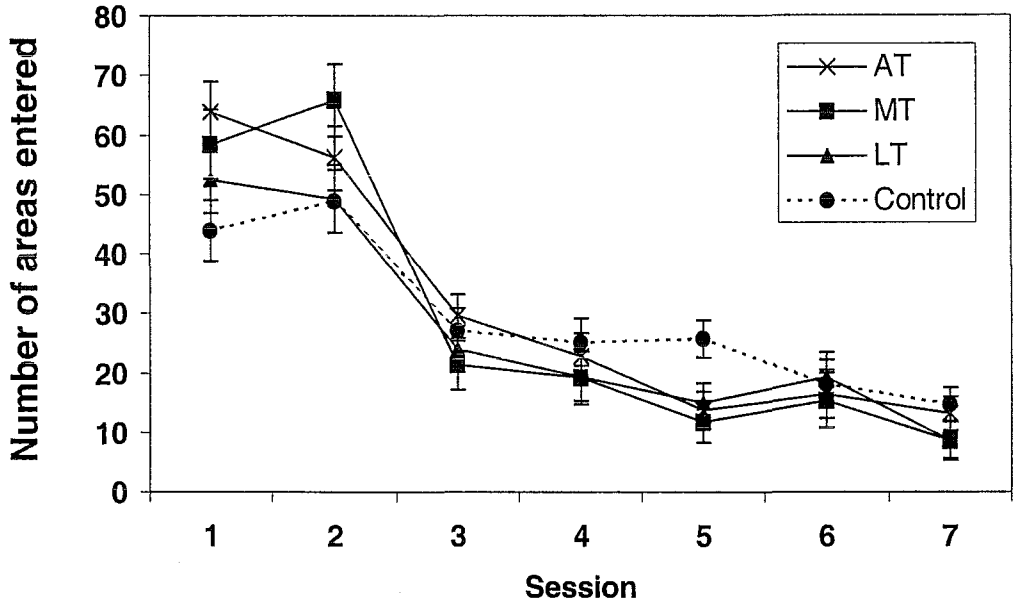


Figure 3.9 Average number of areas entered for the AT, MT, LT and Control groups during each six-minute session in the spontaneous object recognition task. Vertical bars are ±SEM. There were no objects in Session 1. During Sessions 2 to 4 objects remained in the same positions, but in Session 5 one of the objects moved to a position previously occupied by another object, while the object previously in that position moved to a new location. During Session 7 one of the familiar objects was switched for a novel object.

3.5.2 Exploration of objects

Table 3.3 (p79) shows the average exploration time per object during sessions 2 to 7 for the four groups. Table 3.3 shows that, for all groups, exploration of both the displaced objects (DO) and non-displaced objects (NDO) decreased from Session 2 to Session 7 with the largest decrease occurring from Sessions 2 to 3. In support of this, a 4(Lesion) × 6(Session) × 2(Object) MANOVA confirmed a significant effect for Session ($F(5,145)=134.37$, $P<0.0001$). When collapsed over Sessions and lesion groups, the overall exploration of non-displaced objects appeared higher than exploration of displaced objects, and the MANOVA confirmed a significant effect for object ($F(1,29)=7.91$, $p<0.01$). Data for the new object in Trial 7 were not included in the analysis. Not all

of the lesion groups preferred the non-displaced objects to the same extent. For example, while the MT group spent more time exploring the non-displaced objects, the LT group spent more time exploring the displaced objects, and the MANOVA revealed a significant Object × Lesion interaction ($F(3,29)=4.08$, $p<0.02$). The overall level of object exploration did not differ between lesion groups ($F(3,29)=0.86$, $p>0.47$), and there was no Lesion × Session interaction ($F(15,145)=1.33$, $p>0.19$).

Table 3.3 Spontaneous object exploration: Average exploration per object (seconds) for displaced (DO), non-displaced (NDO) objects and the novel object (NO).

	Session 2		**Session 3**		**Session 4**		**Session 5**		**Session 6**		**Session 7**			**Over all Sessions**	
	DO	NDO	DO	NDO	DO	NDO	DO	NDO	DO	NDO	NO	DO	NDO	DO	NDO
AT	9.84 (1.30)	15.32 (1.60)	5.47 (1.33)	5.57 (1.71)	2.74 (0.84)	4.31 (0.95)	2.27 (0.82)	0.45 (0.57)	1.59 (0.83)	2.38 (0.89)	5.82 (2.87)	1.46 (0.39)	3.40 (0.91)	3.90	5.24
MT	10.69 (1.47)	17.45 (1.81)	3.05 (1.51)	5.82 (1.93)	2.84 (0.95)	2.58 (1.08)	0.50 (0.93)	1.13 (0.65)	2.67 (0.95)	3.24 (1.01)	1.43 (3.25)	0.18 (0.44)	0.80 (1.03)	3.32	5.17
LT	14.67 (1.38)	12.49 (1.70)	6.11 (1.41)	4.00 (1.81)	3.29 (0.89)	3.22 (1.01)	3.20 (0.87)	2.60 (0.61)	2.42 (0.89)	2.79 (0.94)	4.26 (3.04)	0.09 (0.41)	0.59 (0.96)	4.96	4.28
C	11.32 (1.30)	12.78 (1.60)	2.62 (1.33)	2.07 (1.71)	3.15 (0.84)	2.09 (0.95)	0.93 (0.82)	2.38 (0.57)	1.16 (0.83)	1.74 (0.89)	6.92 (2.87)	0.95 (0.39)	2.09 (0.91)	3.36	3.86
Average	13.07		4.34		3.03		1.68		2.25		2.33				

Note: Data are means (SEM)

Abbreviations: DO= displaced object (object that is displaced in Session 5); NDO= non-displaced object (object that is not displaced in Session 5); NO= new object (Session 7 only). Sessions 2 to 4= objects remained in the same locations; Sessions 5 and 6= one object moved to a location previously occupied by another object, and the object from the previously occupied location moved to a novel location; Session 7= a new object was introduced in the place of a familiar non-displaced object.

3.5.3 Response to the spatial change of objects

In Session 5, two of the familiar objects were moved to new spatial locations. Exploration times for displaced and non-displaced objects in Session 4 (the last session before the spatial change) and Session 5 (the first session after the spatial change) are provided in Table 3.3 (p79). Figure 3.10 (p81) presents these data in terms of the change in exploration of the displaced and non-displaced objects between Session 4 and Session 5. Exploration of both displaced and non-displaced objects decreased following the spatial change. In support of this, A 4(Lesion) × 2(Object) × 2(Session) MANOVA showed a significant effect for Session ($F(1,29)=11.03$, $p<0.01$). The overall exploration of displaced and non-displaced objects appeared to be similar, and the MANOVA showed no effect for Object ($F(1,29)=0.004$, $p>0.95$). There were no significant interactions for Object × Lesion ($F3,29)=0.14$, $p>0.94$), Object × Session ($F(1,29)=0.04$, $p>0.84$) or Session × Lesion ($F(3,29)=1.08$, $p>0.37$). While the exploration in Session 4 and Session 5 was similar for both displaced and non-displaced objects for the MT and LT groups, the Control group showed a drop in exploration of displaced objects, but a slight increase in exploration of non-displaced objects, while the AT group showed a small drop in exploration of displaced objects, and a much larger drop in exploration of non-displaced objects. In support of this, there was a significant Lesion × Object × Session interaction ($F(3,29)= 4.10$, $p<0.02$).

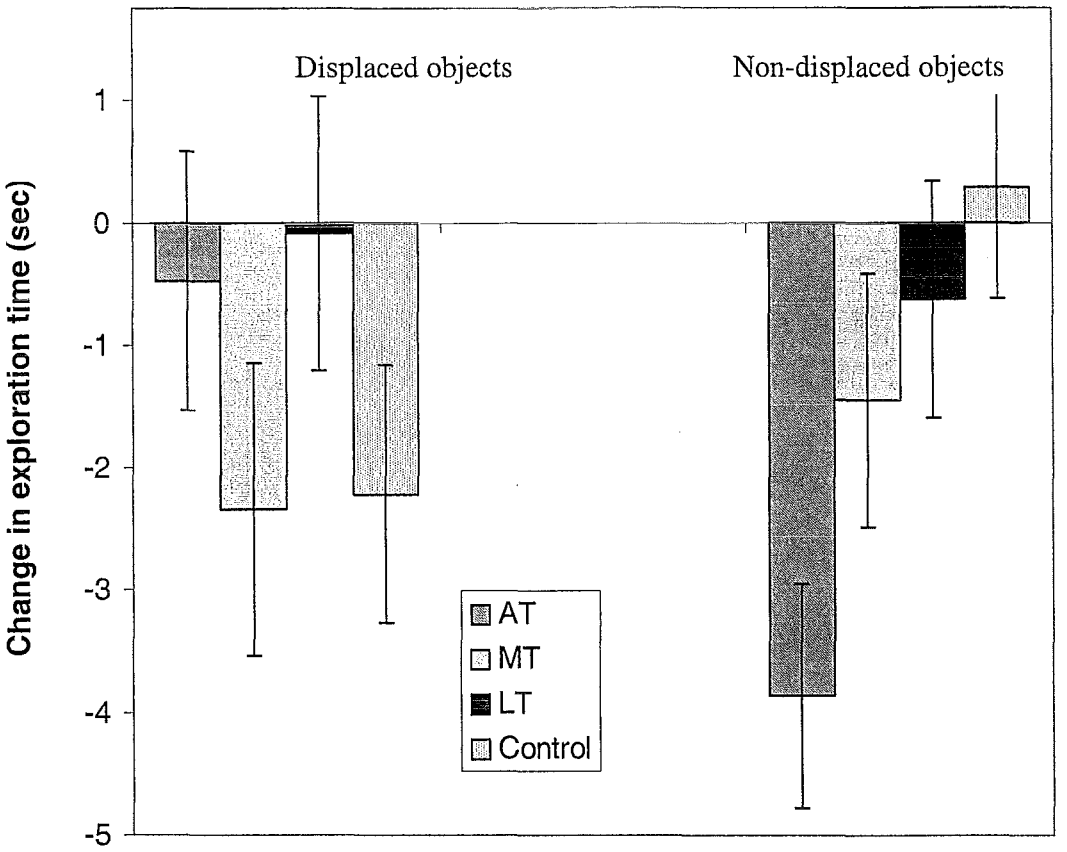


Figure 3.10 Average change in exploration time from Session 4 to Session 5 (when the spatial change occurred) for displaced and non-displaced objects for the AT, MT, LT and Control groups. Vertical bars are ±SEM.

3.5.4 Response to the novel object

In Session 7, one of the familiar objects was switched with a new object. Figure 3.11 (p82) shows the average exploration time per object for the novel and familiar objects for the 4 groups during Session 7. The 'familiar objects' average is the average of the two non-displaced objects only, because following the spatial change that occurred in Sessions 5 the familiarity/novelty status of the displaced objects is somewhat ambiguous. Figure 3.11 shows that only the Control group spent considerably more time exploring the novel object than the familiar objects. Although the AT, MT and LT groups all showed an increase in exploration of the novel objects, error scores were large in these groups and no clear differences in exploration of novel and familiar objects were seen.

A 4(Lesion) × 2(Object) MANOVA revealed that, when condensed over groups, rats spent more time exploring novel objects than familiar objects (main effect for object: $F(1,29)=4.30$, $p<0.05$). Despite the apparent differences between the four groups, there was no significant effect for Lesion ($F(3,29)=0.91$, $p>0.44$) and the Object × Lesion interaction was not significant ($F(3,29)=0.40$, $p>0.75$).

Detection of the object change was also examined using a ratio score, that is, the average exploration time per object for the familiar object minus the average exploration time per object for the familiar objects, divided by the total average exploration time per object for the familiar and novel objects. Ratios were calculated for individual rats and then averaged within lesion groups. Figure 3.12 (p83) shows the average ratio scores for the four groups. While the analysis of the exploration time per object indicated that, when condensed over groups, rats spent more time exploring novel objects than familiar objects, analysis of the discrimination ratios did not support this. Figure 3.12 shows that the discrimination ratios for all groups, including Controls, were close to zero. Independent single-sample t-tests confirmed that none of the groups showed a discrimination ratio that was significantly different to zero (AT: $t(8)=-0.78$, $p>0.45$, MT: $t(6)=-0.73$, $p>0.49$, LT: $t(7)=0.24$, $p>0.82$, Control: $t(8)=0.95$, $p>0.36$).

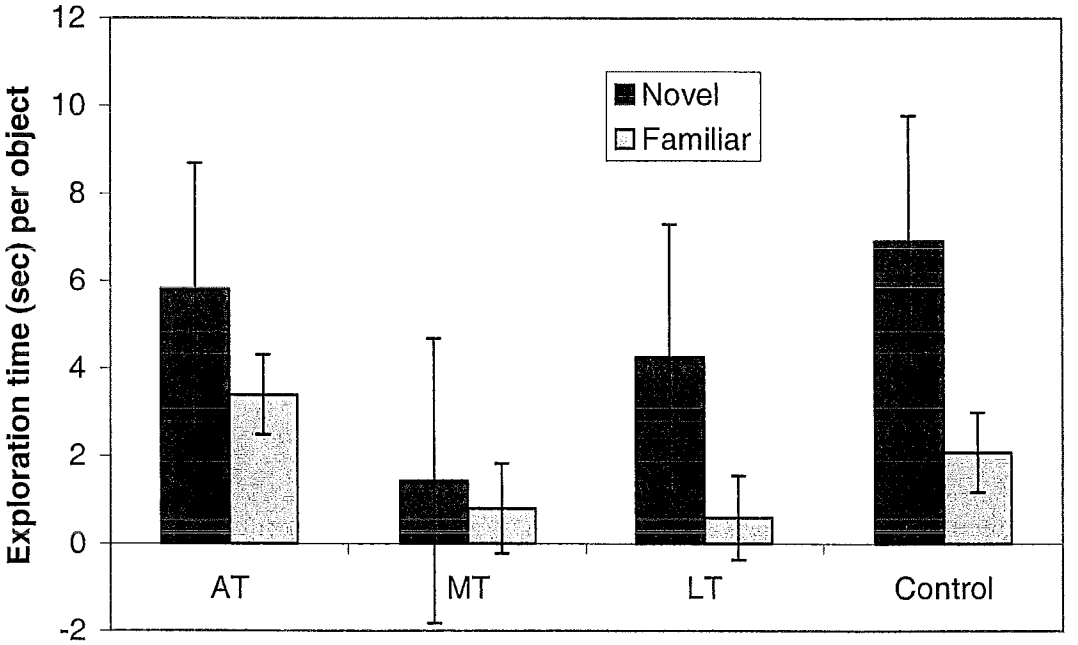


Figure 3.11 Average exploration time per object (sec) for the novel and familiar objects during Session 7 for the AT, MT, LT and Control groups. Vertical bars are ±SEM.

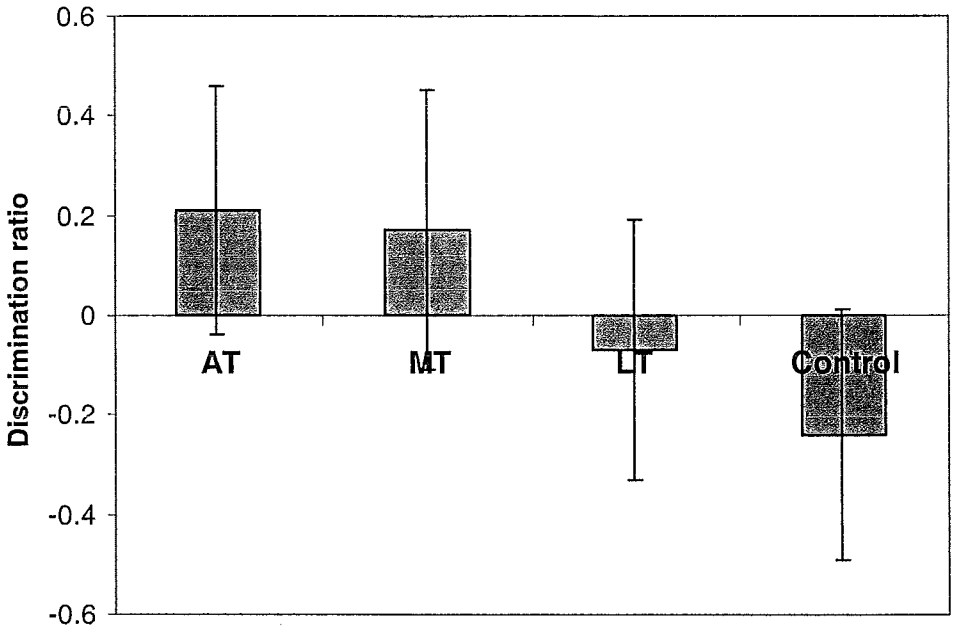


Figure 3.12 Average discrimination ratio (New-Familiar/New+Familiar) for the four groups. Vertical bars are ±SEM.

3.5.5 Overall object preference

The overall level of exploration of each object in the spontaneous object recognition task was examined in order to assess whether animals had an innate preference for some objects over others. Figure 3.13 (p84) shows the average total exploration time per session for the glass vase, glass bottle (these two objects changed position in Session 5), ceramic ornament, plastic bedleg and plastic monkey (note that exploration time for the soap holder is not included as this object was only present in Session 7). The figure shows that rats spent the most time exploring the monkey and bottle, less time exploring the ornament, and the least amount of time exploring the vase and bedleg. The figure shows that, overall, this pattern of preference was similar for the four lesion groups. A 4(Lesion) x 5(Object) MANOVA confirmed these observations. There was a significant main effect for Object ($F(4,124)=29.62$, $p<0.0001$). Post-hoc tests (Newman Keuls) revealed significant differences in exploration time between all objects (all $p<0.05$) except for the monkey and bottle

(p>0.44). There was no significant main effect for Lesion (F(3,31)=0.86, p>0.47) and the interaction was not significant (F(12,124)=1.66, p>0.08).

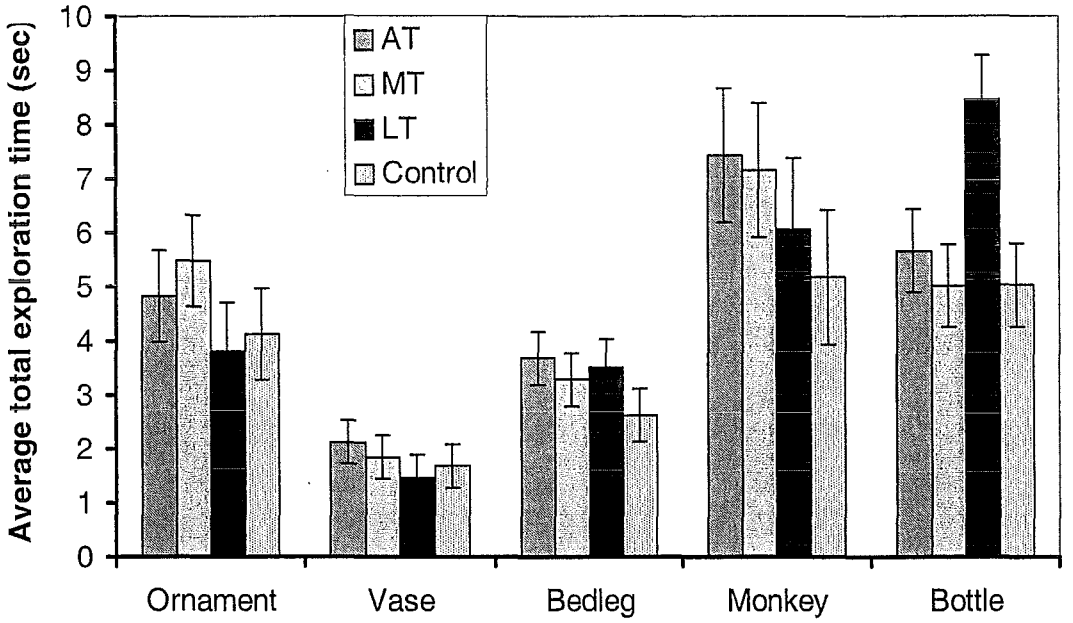


Figure 3.13. Average total exploration time in Sessions 2 to 7 for the ornament, vase, bedleg, monkey and bottle for the AT, MT, LT and Control groups. Note that the data for the soap holder are not included because it was present only in Session 7.

3.6 Lesion-behaviour correlations

3.6.1 Relationship between lesion damage and performance on the odour-place paired-associate task

Figure 3.14 (p85) shows a scatterplot of average latency difference scores on the final week (Week 14) of the odour-place paired-associate task and the percent damage to the AT region across all rats in the 3 lesion groups (note that, in all of the scatterplots provided some symbols may represent more than one rat). While the two MT animals that did not meet our criteria for inclusion were not included in any of the previous analyses, they are included here because it is presumed that there may be a relationship between damage and performance in these rats. The pattern of data in the scatterplot indicates that a simple correlation is inappropriate so a description is provided only. For interest, the Spearman rank order correlation is provided in the legends to the figures. The

scatterplot in Figure 3.14 provides some indication that, within the AT group, performance on the odour-place association task decreases as AT damage increases. There is however very little AT damage in the MT and LT animals and the scatterplot shows no apparent relationship between the amount of AT damage and performance in these groups. Similar scatterplots for the average latency difference in Week 14 and the amount of MT and LT damage are shown in Figures 3.15 and 3.16 (p86). Figure 3.15 suggests that MT damage may actually improve performance, but this may be an artefact of the impairments shown by AT and LT rats. Figure 3.16 shows no obvious relationship between performance in Week 14 and the amount of LT damage.

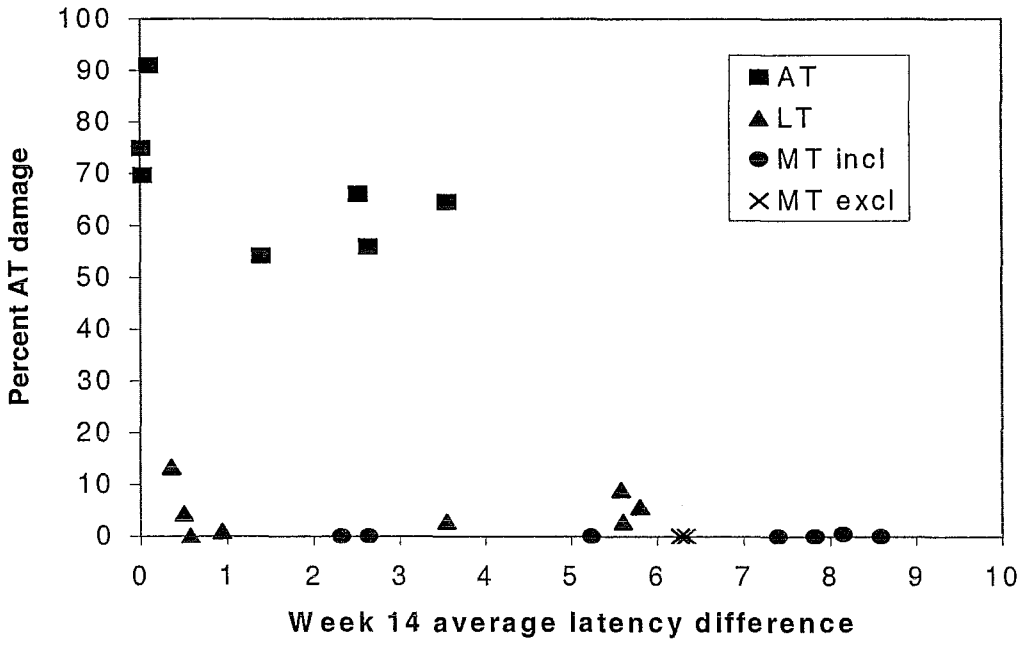


Figure 3.14 Scatterplot of performance on the final week of the odour-place paired-associate task and percent damage to the AT region (Spearman r =-0.69, $p<0.05$).

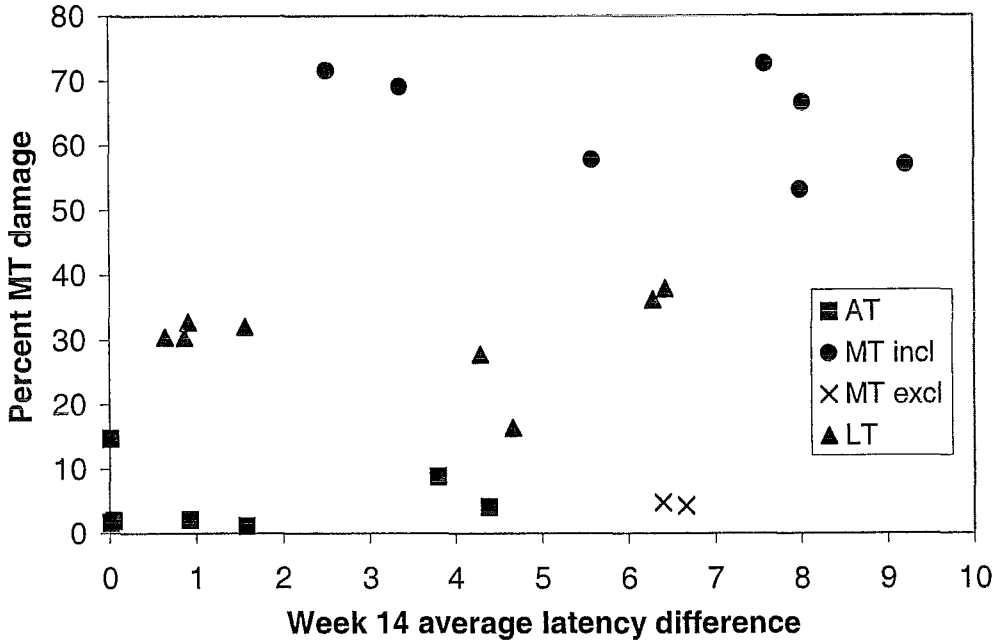


Figure 3.15 Scatterplot of performance on the final week of the odour-place paired-associate task and percent damage to the MT region (Spearman r = 0.57, p<0.05).

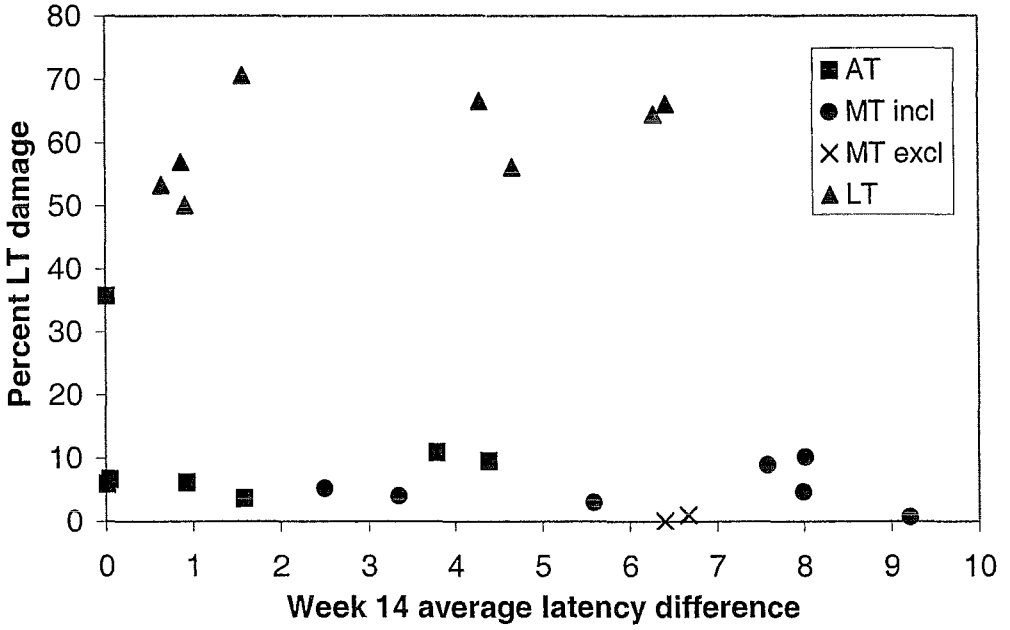


Figure 3.16 Scatterplot of performance on the final week of the odour-place paired-associate task and percent damage to the LT region (Spearman r = -0.25, p<0.05).

The scatterplot of the number of days to criterion on the odour-place paired-associate task and the percent damage to the AT region is shown in Figure 3.17 (this page). The graph shows that there is little obvious relationship between the amount of AT damage and the number of days to criterion within the AT group. Again, there appears to be no relationship between AT damage and days to criterion in the MT and LT animals.

Scatterplots of the number of days to criterion on the odour-place paired-associate task and the amount of MT and LT damage are shown in Figures 3.18 and 3.19 (p88). Again, neither graph shows an obvious relationship between MT or LT damage and the number of days to criterion.

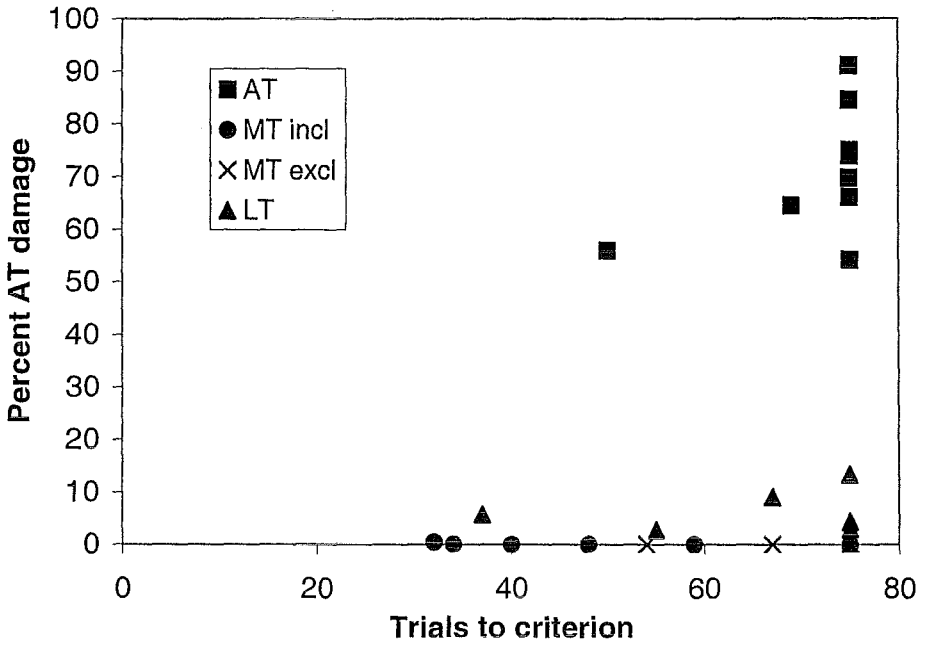


Figure 3.17 Scatterplot of the number of trials to criterion on the odour-place paired-associate task and the percent bilateral AT damage for all rats in the study (Spearman $r = 0.45$, $p<0.05$).

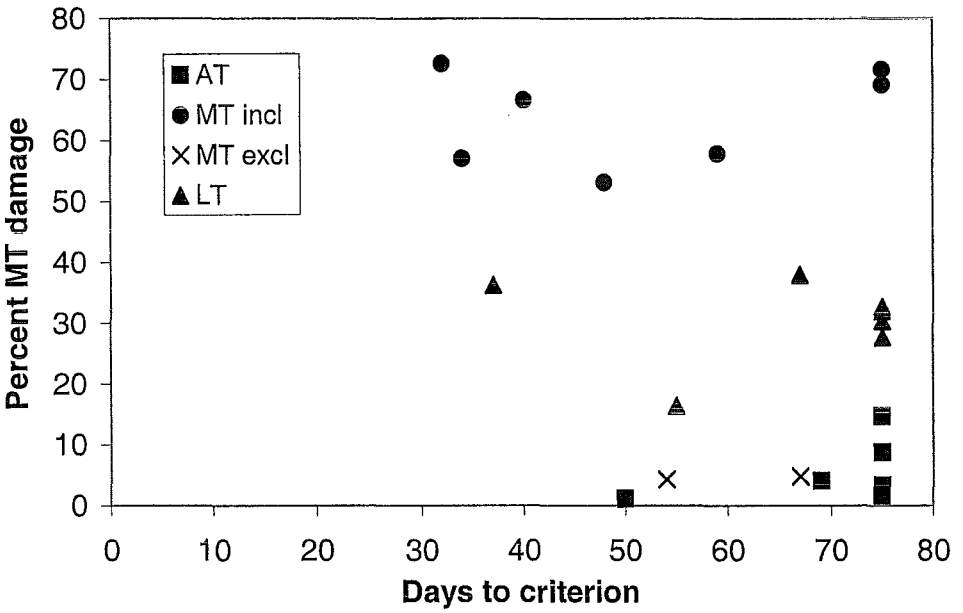


Figure 3.18 Scatterplot of the number of days to criterion on the odour-place paired-associate task and the amount of bilateral MT damage for all rats in the study (Spearman r = -0.35, not significant).

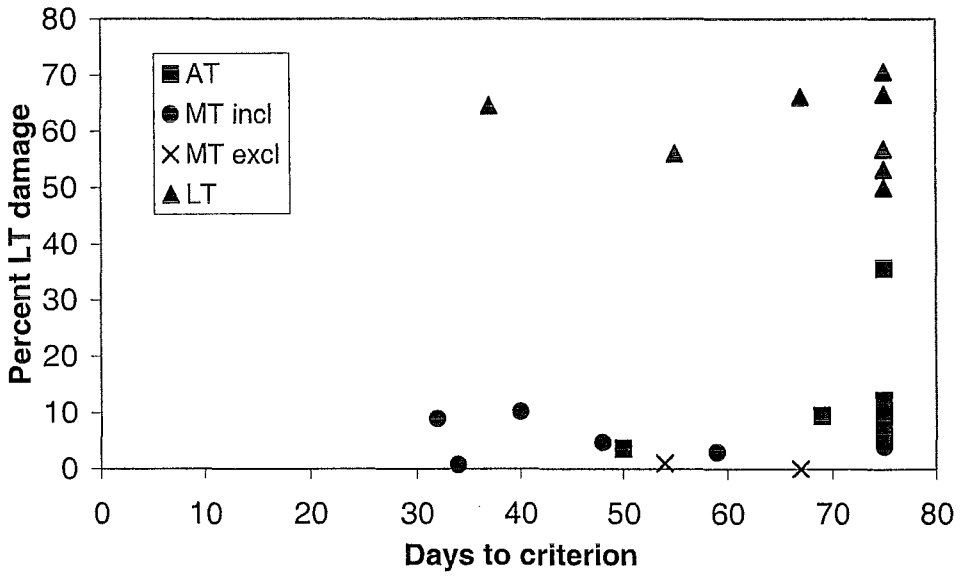


Figure 3.19 Scatterplot of the number of days to criterion on the odour-place paired-associate task and the amount of bilateral LT damage for all rats in the study (Spearman r = 0.30, not significant).

One observation that is apparent from Figures 3.14 (p85) and 3.16 (p86) is that within the group of LT animals, there is a cluster of animals with very poor performance (latency difference scores <1), and another cluster with better performance, although all appear to have similar amounts of little AT damage. Individual behavioural and lesion data were examined to try and determine the source of this difference (Table 3.4, p90). The table shows that the four LT animals with very poor performance (6B, 8R, 10R, 7R) were ranked (within the LT group) 1st, 4th, 7th and 8th for percent AT damage, 5th, 6th, 3rd and 4th for percent MT damage, and 7th, 5th, 8th and 1st for percent LT damage. They did not appear to have large amounts of damage to any of the other structures listed in Table 3.4 relative to the other members of the LT group. Similarly, within the AT group, there is a cluster of animals with latency differences scores of approximately zero, and another cluster with modest latency difference scores. The five animals with very low latency difference scores (1B, 4G, 4B, 3P, 1R) also had the five largest amounts of AT damage within the AT group. They did not appear to have large amounts of damage to any of the other structures listed in Table 3.4 relative to the other members of the AT group. Two members of the MT group showed low average latency difference scores (9R, 4P). Neither of these animals had any AT damage, but they were ranked (within the MT group) 2nd and 3rd for percent MT damage, and 3rd and 5th for percent LT damage. They did not appear to have large amounts of damage to any of the other structures listed in Table 3.4 relative to the other members of the MT group.

Only three of the LT animals had reached criterion in the odour-place paired-associate task by the end of testing. However, in these three rats the average number of days to criterion (53.0 days) was higher than the average for the five MT animals that reached criterion (42.6 days). Only two of the AT animals reached criterion, with a mean of 59.5 days.

Table 3.4 Behavioural data from the odour-place paired-associate task and percent damage to selected areas for individual rats in the study.

		Week 14	DTC	AT	MT	LT	IAM	LD	PT	PVA	PV/ PVP	Re	Rh
AT lesions	1B	-0.1	75	91.0	3.3	12.2	56.9	7.3	21.6	0.1	0.0	3.5	3.6
	4G	0.0	75	84.5	14.7	35.7	66.7	18.3	39.0	0.9	0.0	0.0	4.8
	4B	0.0	75	74.9	1.7	5.9	18.2	2.9	8.2	0.0	0.0	0.0	0.1
	3P	0.0	75	69.7	2.0	6.7	15.0	6.0	6.3	0.0	0.0	2.6	7.1
	1R	0.0	75	74.0	1.7	8.0	7.1	5.0	10.6	0.0	0.0	0.4	0.0
	6R	0.9	75	54.2	2.1	6.1	7.2	6.1	17.7	0.1	0.0	0.0	0.0
	3G	1.6	50	56.0	1.1	3.6	11.6	4.5	7.8	0.0	0.0	0.0	0.0
	5R	3.8	75	66.1	8.8	10.9	57.6	13.7	63.6	46.5	0.0	0.8	8.8
	9G	4.4	69	64.6	4.1	9.5	34.7	8.9	19.4	0.0	0.0	0.0	0.0
MT lesions	9R	2.5	75	0.0	71.6	5.2	0.0	0.0	0.0	1.4	72.0	0.0	0.0
	4P	3.3	75	0.0	69.1	4.0	0.0	0.0	9.7	18.7	51.8	0.0	0.0
	3B	5.6	59	0.0	57.8	3.0	0.0	0.0	0.0	0.1	52.0	0.0	0.0
	5G *	6.4	67	0.0	4.8	0.0	0.0	0.0	0.0	0.0	46.8	0.0	0.0
	2R *	6.7	54	0.0	4.3	1.0	0.0	0.0	0.0	10.7	33.5	0.0	0.0
	3R	7.6	32	0.5	72.6	8.9	4.0	0.0	2.7	10.9	67.6	0.0	0.0
	2P	8.0	40	0.0	66.6	10.2	7.5	0.0	0.0	0.0	53.7	0.0	3.9
	5B	8.0	48	0.0	53.1	4.7	0.0	0.0	0.0	0.0	30.6	0.0	0.0
	6G	9.2	34	0.0	57.0	0.7	0.0	0.0	0.0	0.0	62.0	0.0	0.0
LT lesions	6B	0.6	75	13.3	30.4	53.3	4.2	3.3	1.3	0.0	0.0	0.0	0.0
	8R	0.9	75	0.0	30.3	56.9	0.0	1.4	0.0	0.0	1.2	0.0	0.0
	10R	0.9	75	4.3	32.7	50.1	3.4	8.4	0.0	0.0	0.6	0.0	0.0
	7R	1.6	75	0.9	32.0	70.6	0.7	2.0	0.0	0.0	0.0	0.0	0.2
	9B	4.3	75	2.9	27.7	66.6	0.0	6.2	0.0	0.0	0.0	0.0	0.0
	9P	4.7	55	2.8	16.4	56.1	0.0	1.8	0.2	0.0	0.0	0.0	0.0
	7G	6.3	37	5.7	36.2	64.5	2.6	2.7	0.0	0.0	0.0	0.0	0.5
	7B	6.4	67	9.0	37.9	66.1	0.9	3.1	0.0	0.0	0.0	0.0	0.0

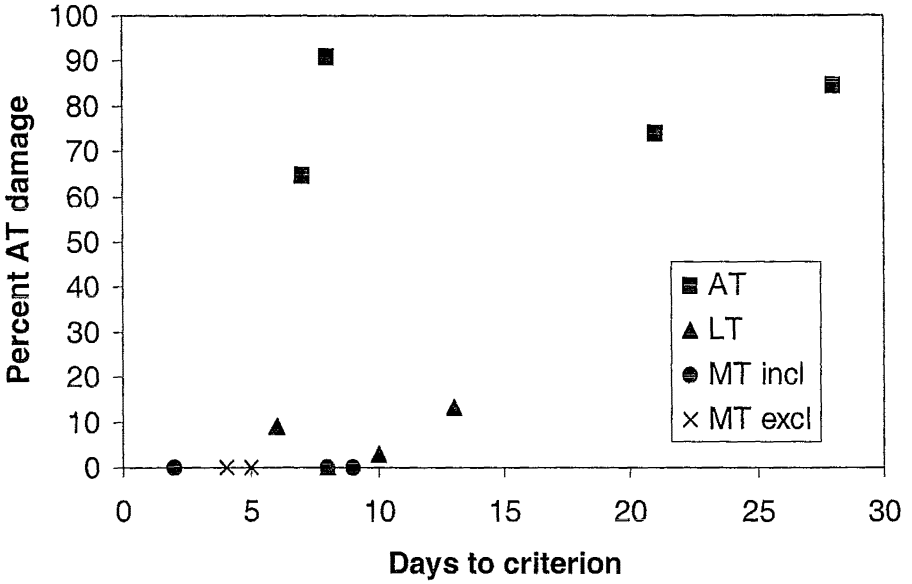
* Lesion did not meet criteria for inclusion in behavioural analysis

Abbreviations: AT= anterior thalamic aggregate; DTC= number of days to criterion in the odour-place association task; IAM= interanterodorsal nucleus; LD= laterodorsal nucleus; LT= lateral thalamic aggregate; MT= posteromedial thalamic aggregate; PT= paratenial nucleus; PVA= anterior paraventricular nucleus; PV/PVP= paraventricular nucleus/posterior paraventricular nucleus; Re= reunions nucleus; Rh= rhomboid nucleus; Week 14= average latency difference score in week 14 of the odour-place paired-associate task.

3.6.2 Relationship between lesion damage and performance on the simple discrimination tasks

Figures 3.20A and 3.20B (p92) show scatterplots of the amount of AT damage and performance on the simple odour discrimination and simple place discrimination tasks. Neither of the figures revealed any apparent relationship between AT damage and performance on the simple discrimination tasks. While two AT animals had a high number of days to criterion on the simple place discrimination task, they did not have particularly high amounts of AT damage relative to the other AT animals. Similarly, the two AT animals that had high days to criterion on the simple odour discrimination task had similar levels of AT damage to the other AT animals.

A



B

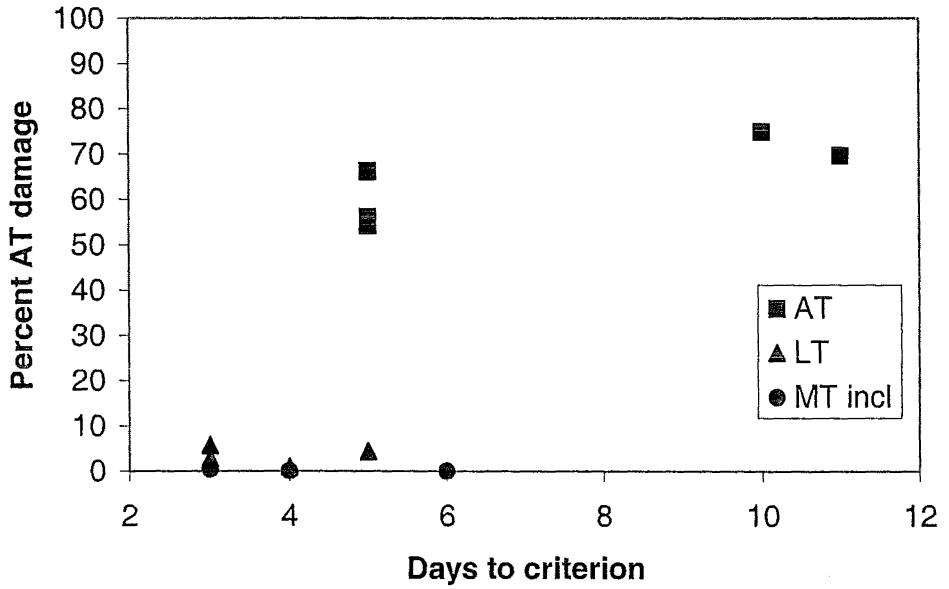


Figure 3.20 Percent bilateral damage to the AT region and performance on the simple place discrimination (A) and simple odour discrimination (B) tasks (Spearman r: odour=0.52, not significant; place: 0.55, not significant).

4. Discussion

4.1 Summary of main findings and issues

A common view of diencephalic amnesia is that there is a single key diencephalic site that responsible for the memory impairment. However, there has been little agreement over where this key site is located, with evidence suggesting various areas including the AT, MD and IL (Harding et al., 2000; Victor et al., 1971; Zhang et al., 1998). An alternative to this traditional view is that there is no single site responsible for diencephalic amnesia. Instead, different diencephalic areas contribute to amnesia in subtly different ways. Recent work at the University of Canterbury has provided support for this latter view, by demonstrating that multiple memory systems may include different regions of the thalamus, with the AT responsible for spatial memory, the MT (that is, the MD region excluding the lateral MD) responsible for reward value memory, and the LT (that is, the IL region, including the lateral MD) responsible for response memory (Mitchell & Dalrymple-Alford, 2004, 2005).

The current study examined the role of these three aggregates of thalamic nuclei (the AT, MT and LT) in odour-place paired-associate learning. Paired associate learning tasks, especially those that involve a spatial component, are regarded as important models of episodic-like memory in non-human animals (Aggleton & Pearce, 2001). According to Kesner's (1998) multiple memory systems model, the odour-place paired-associate task involves pattern association between spatial and odour attributes, with the implicit assumption that space and the hippocampus are critical factors. The current study revealed that AT lesions severely impaired performance on an odour-place paired-associate task. LT lesions also severely impaired performance. Conversely, MT lesions had no effect on performance. While the amount of AT damage caused by the lesion seemed to be related to performance in the AT group, AT damage could not explain the impairments seen in the LT group. In contrast, the LT group was not impaired in either simple odour discrimination or simple place discrimination. The AT group showed some impairment in these simple discrimination tasks, perhaps especially the odour discrimination task. Results for the spatial probe tasks were less clear, but seemed to indicate that the MT and Control groups, especially, used egocentric strategies to some extent to solve the odour-place paired-associate task, although they were not relying fully on these egocentric strategies. The Control and MT groups were able to make use of allocentric cues to solve the task, although the MT group seemed to be more able to do this than the Control group.

The spontaneous object recognition task revealed that no group showed a clear preference for novel objects at least in Poucet's (Save et al., 1992) version of the spontaneous object recognition task. No group showed clear evidence of detecting changes in object-place combinations, although only the Controls showed elevated activity when object-place changes occurred.

Previous evidence has suggested that the hippocampus is involved in pattern association processes, but there is debate over whether it is involved only in pattern associations involving a spatial attribute, or in all pattern associations. In the only previous study to examine the effects of thalamic lesions in a paired-associate task, Sziklas and Petrides (1999) demonstrated that rats with AT lesions were impaired in a paired-associate task that involved an association between an object and a spatial location, but were not impaired when the association was between an object and a left or right body turn. They concluded that the AT is involved in such conditional discriminations only when spatial attributes are involved. No previous study has examined the role of the MT and LT in pattern association processes. Other evidence supports the role of the AT in memory involving a spatial attribute (for examples, see Aggleton et al., 1995; Byatt & Dalrymple-Alford, 1996; Sziklas & Petrides, 1999). While early evidence implicated the MT in spatial memory (Stokes & Best, 1988), more recent studies have found only slight (Hunt & Aggleton, 1998) or no (Burk & Mair, 1998) deficits after MT lesions. Deficits have been found after IL (Burk & Mair, 1998; Mair et al., 1998) and L-IML lesions (Burk & Mair, 1998), although it has been argued that these deficits may reflect a general impairment in learning, rather than a specific spatial memory impairment (Zhang et al., 1998) or impairments brought on by unintentional AT damage (Aggleton & Brown, 1999; Mitchell & Dalrymple-Alford, 2005).

Few studies have examined the role of the thalamic nuclei in memory involving odour attributes and the evidence is again mixed. It has been suggested that the MD may be involved in odour memory due to its connections with cortical and sub-cortical odour areas (Eichenbaum et al., 1980; McBride & Slotnick, 1997). MD lesions can impair performance on odour memory tasks, especially when odour serial reversal learning is involved (Eichenbaum et al., 1980; McBride & Slotnick, 1997; Zhang et al., 1998). L-IML lesions have also been shown to impair odour memory (Koger & Mair, 1994; Zhang et al., 1998), but as mentioned previously, this may be due to a general learning impairment rather than an odour memory impairment *per se*. No study thus far has examined the effects of AT lesions on odour memory.

Several studies have examined the role of the thalamic nuclei in spontaneous object recognition tasks. Thus far, no study has shown that thalamic lesions impair the recognition of novel objects.

A discussion of the main findings and issues from each task follows.

4.1.1 Odour-place paired-associate task

Rats were trained for 14 weeks in a go/no-go odour-place paired-associate task. By the end of the 14 week period, the MT and Control groups had reached a similarly high level of performance. The LT group was impaired relative to the MT and Control groups, although they did show slight improvement towards the end of the 14 weeks. The AT group was the most severely impaired, and showed virtually no improvement over the 14 week training period.

The AT group's severe impairment relative to the Control group on the odour-place paired-associate task is consistent with previous evidence showing that the AT are involved in pattern association when it involves a spatial attribute (Sziklas and Petrides, 1999). The very small increase in performance of the AT group over the 14 weeks can be accounted for by a modest increase in performance for two AT rats only. Other research has also indicated that AT lesions impair performance on standard, non-associative spatial tasks (Alexinsky, 2001; Gaffan et al., 2001; Mitchell & Dalrymple-Alford, 2005; Moran & Dalrymple-Alford, 2003; Sziklas & Petrides, 1999; Ward-Robinson et al., 2002). Lesions to the hippocampus have also been shown to impair performance on pattern association tasks involving a spatial attribute. Specifically, large hippocampal lesions and lesions of the CA3 layer of the hippocampus have been shown to impair performance in object-place and odour-place pattern associations, while lesions to the CA1 or dentate gyrus do not impair performance (Gilbert & Kesner, 2002, 2003). The similarity of the effects of hippocampal and AT lesions on memory is consistent with Aggleton and Brown's (1999) suggestion that the AT and hippocampus are both part of an 'extended hippocampal system' that also comprises the fornix and mammillary bodies, and is responsible for recall memory. The results of the current study provide further support for the existence of this system. While some evidence suggests that the hippocampus is only involved in pattern associations that contain a spatial component (Gilbert & Kesner, 2002), other evidence suggests that the hippocampus is also involved in non-spatial pattern association (Alvarez et al., 2002; Bunsey & Eichenbaum, 1993). It is not clear from the current study whether the AT are involved in all pattern association processes or just those

that involve a spatial component, although a previous study has demonstrated that AT lesions do not impair performance on an object-response (body turn) paired-associate task (Sziklas and Petrides 1999). Further research examining the role of selective AT lesions in pattern association tasks involving different attributes would provide evidence regarding the role of the AT in non-spatial pattern association processes. It would also provide evidence on the similarity, or possible difference, of AT and hippocampal lesions. Examining the effects of AT lesions on an object-odour paired-associate task would be particularly valuable because it would provide information on the role of the AT in pattern associations that do not involve spatial attributes.

The LT group was also impaired on the odour-place association task, although they showed a slight improvement in performance towards the end of the training period. This improvement reflected the fact that some LT rats were beginning to acquire the odour-place association, although the number of days required to reach criterion on this task (that is, in those animals who reached criterion by the end of training) was greater in the LT group than in most MT and Control rats. Though no previous study has examined the role of the LT in pattern association, deficits on tasks involving a spatial component after L-IML and IL lesions have been reported previously (Burk & Mair, 1998; Harrison & Mair, 1996; Mair et al., 1998; Porter et al., 2001). However the only previous studies to use highly selective lesions to the same LT aggregate used in the current study found that these lesions did not impair performance in the radial maze (Mitchell & Dalrymple-Alford, 2004, 2005). Because highly selective LT lesions do not seem to impair performance on "simple" spatial tasks, the deficit seen in the current study may be due to the pattern association processes involved in the odour-place paired-associate task. It is possible that LT lesions impair pattern association that involves a spatial attribute, but not simple spatial tasks that do not involve pattern association. Another possibility is that lesions to the LT produce a general impairment in learning that may be related to attention or executive function. However, some general impairment should be evident on all memory tasks, and the current study demonstrated that LT lesions did not impair performance on simple discrimination tasks. Other studies have also demonstrated that LT lesions do not impair performance on all memory tasks and there is no evidence of deficits in "attention" tasks (for example, see Mitchell, 2004; Mitchell & Dalrymple-Alford, 2005; Newman & Burk, 2004; Savage et al., 1998). Further research is required to examine the role of the AT and LT in pattern association processes, including those that involve spatial and non-spatial attributes.

In contrast to the AT and LT groups, the MT group was unimpaired relative to Controls on the odour-place paired-associate task. Again, no previous study has examined the effects of MT

lesions on pattern association processes, but previous studies have shown the MT lesions do not impair performance on non-associative spatial tasks (Burk & Mair, 1998; Mitchell & Dalrymple-Alford, 2005). These previous studies and the present study are inconsistent with the original results of Stokes and Best (1988) and to a lesser extent those of Hunt and Aggleton (1998) and Alexinsky (2001). It has been suggested that the method used to produce the lesion may account for some of the inconsistencies in results. Stokes and Best (1988; 1990a) used electrolytic lesions, which destroy both cell bodies and fibres of passage, whereas Burk and Mair (1998), Mitchell and Dalrymple-Alford (Mitchell & Dalrymple-Alford, 2005) and the current study used NMDA lesions, which destroy cell bodies but spare fibres of passage. However, other studies (including a further study by Stokes and Best) using NMDA or ibotenic acid lesions (Alexinsky, 2001; Hunt & Aggleton, 1998; Stokes & Best, 1990b) have reported deficits in spatial memory, suggesting that the method used to produce the lesion cannot account for all of the inconsistency in results. Hunt and Aggleton (1998) have suggested that MD lesions only produce deficits when the task is sufficiently demanding. The current study does not provide support for this suggestion, as it demonstrated that MT lesions do not impair performance on a clearly demanding object-odour paired-associate task. Another possibility is that lesions to the MD may extend slightly into the adjacent AT region. Lesions to the AT cause severe deficits on spatial tasks, and some studies have demonstrated that even very small lesions to this area can produce an impairment (Aggleton et al., 1996b; Byatt & Dalrymple-Alford, 1996; Hunt & Aggleton, 1998). Hunt and Aggleton (1998) reported that MD lesions produced only a slight impairment on the radial arm maze task, but this impairment was greatly increased when damage extended to the AT. They subsequently concluded that unintentional damage to the AT may account for much of the variability in the effects of MD lesions on spatial learning and memory. It is possible that encroachment into the AT may also be responsible for the deficits seen in the study by Alexinsky (2001), although the details regarding these lesions are not available (the issue of lesion specificity and analysis is further discussed in Section 4.1.4). In comparison, the MT lesions in the current study produced almost no damage to the AT region, which may account for the lack of impairment on the odour-place paired-associate task. Previous studies have demonstrated that carefully localised MT and MD lesions that cause little damage to the AT do not impair performance on spatial tasks (Hunt & Aggleton, 1998; Mitchell & Dalrymple-Alford, 2005).

The effects of MT lesions can also be considered in relation to the odour attributes involved in the task. Previous studies have shown that the MT seems to be involved in odour memory only when the task involves serial reversal learning. It has been suggested that the deficits seen in serial

reversal learning are not due to the relative complexity and high cognitive load of the task, but to the specific 'reversal' requirement of the task. The current study provides support for this latter interpretation, as the odour-place paired-associate task is a relatively complex task, but performance was not impaired by MT lesions. Therefore, deficits in olfactory serial reversal learning are more likely due to the specific requirement that animals learn to change their response pattern to take account of a reversal in stimulus-reward associations.

The present study demonstrated that lesions to the AT, MT and LT have different effects on odour-place paired-associate learning. Along with previous work from the University of Canterbury, this provides further support for the idea that descriptions of multiple memory systems in the brain should include the thalamus, and provides some support for a thalamic role in models of memory function, such as that of Kesner (1998). However, while Kesner's (1998) model specifies a number of different brain areas involved in memory, there is no mention of the role of medial thalamus. The current study, along with previous research, suggests that this and related memory models may need to be revised to also take account of the role of the thalamic nuclei in memory, and particularly the role of the AT, MT and LT in pattern association processes.

4.1.2 Spatial probe task

At the conclusion of the odour-place paired-associate task, a set of probe trials was introduced. These probe trials were designed to emphasise the potential involvement of egocentric and allocentric strategies. The manipulation involved the start position being moved to the opposite side of the board on half of the trials. Performance of the LT, MT and Control groups was disrupted by the introduction of probe trials where the start box was switched to the opposite side of the board. As the rats in the AT group already performed poorly on the original task, the little change in performance after introduction of the probe trials is of little consequence. However, an examination of individual data (see Appendix B) indicated that the two AT rats who had showed some evidence of learning in the odour-place paired-associate task showed a drop in performance after the introduction of the probe trials. For the MT, LT and Control groups, the introduction of the spatial probe trials caused a larger drop in performance on new start position trials than old start position trials ("latency difference" drops, in seconds, of 2.57 (MT), 1.47 (LT) and 2.54 (Control) on the old start box position; drops of 4.88 (MT), 2.22 (LT) and 4.92 (Control) on the new start box position, see Table 3.2, p82). While the drop in performance on both the new and old start position trials indicated that the introduction of the probe trials caused a general disruption in behaviour, the larger

drop on new start position trials indicated that rats were having more difficulty solving the task from the new start position than the old. A possible explanation for this is that rats were relying to some extent on egocentric (body) or directional (landmark) cues to solve the odour-place paired-associate task, and reliance on these cues was inappropriate when the start box was switched to the new position. Over the three weeks of probe testing, performance improved overall, especially on the new start position until there was little difference between performance on the new and old start positions, indicating that rats were able to adapt their strategies to take account of allocentric cues. The MT and LT groups regained levels of performance similar to those seen before the introduction of the probe trials. However, the Control group did not manage to regain their previous level of performance. The evidence of the use of egocentric cues to solve the task is consistent with previous evidence that shows that animals may initially learn place solutions to spatial tasks, but with further training they shift to response solutions (Packard & McGaugh, 1996). However, it is unclear why Control animals were unable to return to their previous level of performance after the introduction of the probe trials, whereas MT animals, who had performed similarly to Controls before the probe trials, recovered their previous level of performance. One possibility is that Control animals were relying on egocentric strategies to a greater extent than the MT animals, possibly because MT animals have more difficulty learning egocentric responses than Controls. Previous research has shown that LT lesions, but not AT lesions, impair memory for egocentric response (Mitchell & Dalrymple-Alford, 2004), but that study did not determine if MT lesions do the same.

The finding that rats use a mixture of egocentric and allocentric strategies to solve the odour-place paired-associate task raises the interesting possibility that the deficits of the AT and LT groups on the odour-place paired associate task may reflect different types of impairment. One possibility is that the AT lesions impaired allocentric memory, while LT lesions impaired egocentric response memory. Previous studies have shown that AT lesions impair pattern association involving allocentric, but not egocentric spatial attributes (Sziklas and Petrides 1999) and that LT, but not AT, lesions may impair egocentric memory (Mitchell and Dalrymple-Alford 2004). However, even the Control animals in the current study appeared to be relying on egocentric cues to some extent, suggesting that the impairments seen in the lesion groups are unlikely to be this straightforward. There may be additive, interactive or opposing effects of impairment in egocentric and allocentric spatial memory in these groups. Another difficulty for this suggestion is the question of why AT rats were not able to solve the task by using egocentric strategies, and LT rats by using allocentric strategies, so presumably the associative nature of the task must play some additional role. Clearly

further research is required to examine the specific nature of the deficits in spatial memory seen after AT and LT lesions. Possibilities for further research could include other pattern association tasks involving combinations of spatial, non-spatial and egocentric attributes. An object-odour paired-associate task would provide information regarding the role of the AT and LT in non-spatial and non-egocentric pattern association processes. Pattern association tasks involving egocentric attributes, such as object-response or response-place tasks would also provide valuable information regarding the relative role of the AT, LT and MT in pattern association involving egocentric response.

The finding that animals were relying to some extent on egocentric strategies also suggests that other studies that involve a spatial component may need to control for the use of egocentric strategies in their animals. The previous odour-place paired-associate learning studies by Gilbert and Kesner (2002; 2003) did not examine the use of egocentric strategies to solve the task, so it is possible that animals in this task were relying partly or fully on egocentric strategies, rather than allocentric spatial memory *per se*. Future studies examining spatial memory should take steps to ensure that animals do not use egocentric strategies. For example, the use of egocentric strategies may contribute to inconsistent results in studies using radial arm maze tasks, but the use of these strategies can be minimised by using doors and delays (for example, see Moran & Dalrymple-Alford, 2003). The current odour-place paired-associate task could be similarly improved by incorporating procedures that discourage egocentric strategies. Further studies could investigate the effects of introducing different start positions early in training in order to prevent the use of egocentric strategies. One possibility is a pilot experiment using two groups of control animals, one trained with several different start positions and one trained from only a single start position. The introduction of probe trials (similar to those included in the current experiment) after acquisition of the task would determine whether training on several different start positions encourages animals to rely more on allocentric cues, and therefore causes less of a disruption when probe trials are introduced. If that is the case, then such a modified procedure could be used to reassess the effects of AT, MT and LT lesions.

4.1.2 Simple discrimination tasks

At the conclusion of testing all animals were tested on an odour or spatial discrimination task to determine whether deficits on the odour-place paired-associate task were due to an inability to distinguish between the places or odours or an inability to withhold responses. All of the animals

were able to acquire the task in considerably shorter time than they had taken to acquire the odour-place paired-associate task. This indicated that any impairments in the odour-place paired-associate task were not due to simply discrimination deficits or an inability to withhold responses. It should be noted however, that AT animals took significantly more trials to reach criterion relative to the other three groups, especially on the odour discrimination task. Although AT animals learned the discrimination tasks more slowly than other groups, they still acquired the spatial discrimination task in an average of 16 days, and the odour discrimination task in an average of 7.2 days, compared to the odour-place paired associate task where they were trained for 70 days and showed little or no improvement. Some of the 70 days training on the odour-place paired-associate task would be initial acquisition and familiarization with the task procedures. It is possible that the deficits seen in the AT group on the simple discrimination tasks stem from this group's failure to acquire the original task. Conversely, the LT, MT and Control groups may have benefited from acquisition of the original task, and may have transferred their skills to the simple discrimination tasks. However, after switching to the simple discrimination tasks there would also be an initial period where responses must be modified to suit the changed task requirements. It is unclear from the current study whether animals were able to transfer their learning from the odour-place paired associate task to the simple discrimination tasks. Further research could examine the extent to which acquisition of an associative learning task aids performance on simple discrimination tasks. In the current study the impairments seen in the AT group on the odour-place association task may be partly, but not totally, accounted for by a difficulty in discriminating the odours and places used or difficulty in withholding responses on non-rewarded trials. The latter interpretation seems unlikely given that AT rats were to learn to inhibit responding on both the odour and spatial discrimination tasks. Previous research has shown the hippocampal lesions do not impair performance on simple odour discrimination and simple place discrimination tasks. Thus, the results of the current study contrast with Aggleton and Brown's (1999) proposal that the effects of AT and hippocampal lesions are the same, by suggesting possible point of difference between the effects of AT and hippocampal lesions. However, further work with rats trained on simple odour or place discrimination tasks, with direct comparison to hippocampal or fornix lesions, is needed to answer that question.

Although it was predicted that LT animals would be impaired in all tasks due to the LT possibly playing a general role in learning and memory (see Section 1.9), the LT rats were not impaired on the simple discrimination tasks. This finding does not support the view that the LT play a general role in learning and memory, and instead suggests that the LT play a specific role in

memory that may be tied to one or more processes (such as pattern association) or attributes (such as space or response). While the current study indicated a role for the LT in pattern association processes involving space and odour attributes, further research is required to determine the role of the LT in other types of pattern association, including odour-object, odour-response and place-response paired-associate tasks.

4.1.3 Spontaneous object recognition task

Rats were tested in a series of spontaneous object recognition tasks developed by Poucet (Save et al., 1992) that examined the recognition of novel and familiar objects and object-location combinations. The current study failed to find clear support for Poucet's results. No lesion group showed a clear preference for novel objects, nor showed clear detection of changes in object-place combinations. This contrasts with Poucet's finding that both Control and parietal cortex lesion animals preferentially explore novel objects and are able to detect object-place changes.

All groups showed habituation to the objects, indicating that they clearly were attending to the objects, so any deficits in object or object-place recognition were not due to a failure to attend to objects. Habituation was evident in their decreased movement around the apparatus and decreased exploration of the objects in later sessions compared to earlier sessions. There was no overall difference in locomotion or exploration between the groups, although unlike the other three groups the Control group showed no decrease in locomotion between trials 4 and 5 (when the spatial change occurred).

Although the Control group appeared to show increased exploration of novel objects during Session 7, standard errors were large and post-hoc tests indicated no significant differences between the groups so the reliability of this effect is somewhat uncertain. Analysis of discrimination ratios indicated that no group showed a reliable preference for the novel object over the familiar object, although the use of per object exploration times to calculate these ratios may mean that the ratio analysis is not strictly appropriate. The inconsistency of effects from these two different analyses calls into question the validity of using raw exploration times rather than ratio scores to analyse spontaneous object recognition data, as the raw data may not reflect a true difference in discrimination ratio. Previous research has not found deficits in object recognition following AT, MT or LT lesions (see, for example, Aggleton et al., 1995; Mitchell & Dalrymple-Alford, 2005; Moran & Dalrymple-Alford, 2003). Aggleton and Brown (1999) have suggested that spontaneous

object recognition relies on a 'recognition' memory system that includes the PRC and MD. The current finding that MT lesions impair detection of novel objects thus supports this aspect of their model, although it contrasts with other studies that have failed to find spontaneous object recognition deficits after MT lesions (Hunt & Aggleton, 1998; Mitchell & Dalrymple-Alford, 2005). However, the inconsistency between raw exploration times and ratio scores and the apparent impairment in the Control group in the current study means that further replication of this spontaneous object recognition task is required to clarify these findings.

None of the lesion groups nor the Control group showed clear increases in exploration of the displaced or non-displaced objects following the spatial change. The only indication that the Control group may have detected the spatial change was that they showed no drop in locomotion in Session 5, whereas the AT, MT and LT groups all showed a continuing drop in locomotion. However, exploration of the displaced objects, or exploration in general, did not increase in the Control group following the spatial change. The only previous study to examine the effects of thalamic lesions on spontaneous object-place recognition in rats (Wilton, Baird, Muir, Honey, & Aggleton, 2001) found that combined lesions of the AD and laterodorsal nucleus (LD) impaired recognition of changes in object-place combinations in a spontaneous object recognition task. However, in contrast to the current study control animals in the Wilton et al study were able to detect these changes. The current findings also contrast with those of Poucet (Save et al., 1992), who found that both control animals and animals with parietal cortex lesions increased exploration of the displaced objects. While the current study used the same experimental procedure as Poucet, there are some technical differences that may account for the variation. Firstly, the apparatus used in the current study differed slightly from that used by Poucet- the current apparatus was slightly larger and had transparent walls instead of opaque, and the objects were in slightly different locations. Secondly, Poucet used naïve male Long-Evans hooded rats, whereas the current study used female PVGc hooded rats that had been trained for approximately 17 weeks on a similar apparatus in the experimental room. Finally, while the rats in the current study were given free food for several days prior to the spontaneous object recognition task, their weight was still below free-feeding weight at the time of testing. In contrast, the rats used by Poucet had access to free food at all times. The level of food deprivation of the rats in the current study, combined with the previous experience of receiving food rewards during testing, may have interfered with their performance on the task. Another possibility is that the results were confounded by the innate preference that animals had for some of the objects. While efforts were made to select objects of approximately equal size that could not be easily climbed into or on

to, animals showed a preference for some objects over others. Future studies using object recognition tasks should take care to select objects that are equally preferred by the animals, by conducting pilot preference tests prior to spontaneous object recognition testing. Another alternative is to carefully control for object preference in the analysis by developing and using ratio scores. Comparing the ratio of novel versus familiar object exploration rather than analyzing the raw exploration time may help to control for difference in object preference. With Poucet's spontaneous object recognition task this is slightly problematic, as there are unequal numbers of familiar and novel objects, and a 'per object' exploration time must be used to calculate the ratio. This differs from the raw exploration times used to calculate exploration ratios in previous studies (see, for example Dix & Aggleton, 1999; Moran & Dalrymple-Alford, 2003). Developing a new ratio measurement that can take account of different numbers of objects could minimize the effects of object preference.

4.1.4 Specificity of lesions

There is considerable variation between studies in the location, size and specificity of lesions produced. Lesions often damage non-target areas and this unintentional damage may have an impact on the behavioural deficits observed. Yet few previous studies have performed a detailed quantitative analysis of their lesions. The standard approach is to list damaged areas or describe a 'typical' or 'representative lesion' and provide qualitative statements about the amount of damage to other brain regions and, moreover, the impact of any damage on behaviour. The current study attempted to use highly specific lesions to three thalamic aggregates and quantified the damage to each of these aggregates and several other adjacent areas. The current lesions were very well localised: the median amount of target damage in the AT, MT and LT groups was well above the 50% criterion and the overlap with a non-target thalamic aggregates was minimal. While most lesions also damaged other areas such as the laterodorsal thalamic nucleus and the interanteromedial nucleus, the amount of damage was usually minor or negligible. Moderate amounts of extra damage occurred to the MT for LT lesions (median 30.4%), the central section of the MD for LT lesions (median 69.0%) and the paraventricular nuclei for MT lesions (median 53.7%).

The quantitative analysis has the advantage of allowing the detection of any relationship between the amount of damage and performance on a given task. While the distribution of lesion data in the current study meant that a correlational analysis was not strictly appropriate, within the

group of AT animals, higher levels of AT damage seemed to be related to poorer performance on the odour-place paired-associate task. An important finding was that, within the group of LT animals, deficits on the odour-place paired-associate task did not seem to be related to AT damage. Animals in the LT group typically had minimal amounts of AT damage. Thus, in the current study AT damage is not the cause of the deficits in LT animals, as has been suggested in a previous study using the radial maze (Mitchell and Dalrymple-Alford, 2005). Also, performance in the odour-place paired-associate task in the current LT group did not seem to be related to the amount of LT damage sustained or to damage to any of the other areas studied. One possibility that could account for the deficits in LT animals is that the LT group was slower to acquire the task than the MT and Control groups. Hence some animals had begun to acquire the task whereas others had not. This would mean that the measurement of performance at Week 14 is not a measure of final performance, as it is for the MT and Control groups, but rather a measurement taken during task acquisition.

4.2 Contributions and future directions

The present study makes several contributions to the analysis of the role of the thalamic nuclei in memory processes. The first of these concerns the size, specificity and analysis of lesions to thalamic structures. Building on recent work in our lab at the University of Canterbury, the current study demonstrated that, although the small size and close proximity of thalamic regions means that damage to adjacent areas is inevitable, it is possible to produce highly selective excitotoxic lesions within the thalamus that produce little overlap between lesions and minimise damage to other structures. The current lesions are the most selective we have produced thus far. A quantitative analysis of lesion damage allows for an examination of the relationship between both intentional and unintentional damage and performance on the tasks. Reducing the overlap between lesions and performing a quantitative analysis of lesion damage allows a clearer picture of brain-behaviour relationships to emerge.

The current study provided new evidence on the relative effects of AT, MT and LT lesions in odour-place paired-associate learning. Tasks that involve paired-associate learning are presumed to reflect episodic-like memory in non-human animals. The odour-place paired-associate task used in the current study is a novel task designed and used previously by Kesner (Gilbert and Kesner 2002; 2003). Paired associate learning tasks such as this fulfill the need to have relatively simple models of episodic-like memory that can be used with animals. Because of the number of limitations in

studying the neural basis of episodic memory in humans (see Section 1.2.1 for discussion of this issue), animal models of episodic-like memory are extremely valuable. There is a need for replication and extension of this odour-place paired associate task. While the current study has revealed that both the AT and LT are involved in odour-place paired-associate learning, it is not clear whether the AT and LT are involved in all pattern association processes or only those that involve spatial or response (egocentric) attributes. This issue could be clarified by examining the pattern of impairments would be seen on other paired associate tasks, such as object-place and object-odour paired-associate tasks. An odour-object paired-associate task would be particularly valuable because it could provide information about the role of the AT and LT in pattern associations that do not involve a spatial or egocentric component (an object and pot of scented sand are placed directly in front of the start box).

Another contribution of the current study was the inclusion of spatial probe trials following the odour-place paired-associate task. Previous studies of spatial memory generally have not examined the use of egocentric strategies to solve the task. However, it is important to distinguish between these strategies, as studies have shown that AT lesions may impair allocentric learning but spare egocentric learning (Sziklas & Petrides, 1999), while LT lesions impair egocentric learning but spare allocentric learning (Mitchell and Dalrymple-Alford, 2004). The use of egocentric strategies to solve what are presumed to be allocentric spatial tasks may have masked deficits in previous studies of spatial memory. For example, Berracochea et al (1989) failed to report deficits in radial maze learning after AT lesions, although many previous studies have reported that AT lesions severely impair spatial memory (Aggleton et al., 1995; Byatt & Dalrymple-Alford, 1996; Sziklas & Petrides, 1999). A possible explanation for their failure to find a deficit is that the radial maze that they used lacked doors, allowing animals to rely on egocentric strategies like simply choosing the arm immediately to the right. The current study has also led to the suggestion of modified training schedules during odour-place paired-associate acquisition (see Section 4.1.1). However, an unresolved issue from the current study concerns the extent to which AT, MT and LT lesions affect the use of egocentric and allocentric strategies in spatial tasks. The current study indicated that rats may rely somewhat on egocentric strategies to solve spatial tasks, although some animals can adjust to using allocentric strategies when necessary. Control animals did not adjust to using allocentric strategies as quickly as the MT animals. One possibility that could explain this finding is that MT animals have impaired egocentric memory, and therefore were relying more on allocentric cues than Control animals. Previous research has indicated that LT lesions impair memory for egocentric

response (Mitchell & Dalrymple-Alford, 2004), but thus far no study has examined the role of the MT in memory for egocentric response. One possibility raised by the current study is that AT animals were impaired on the odour-place paired-associate task due to deficits in allocentric memory, while the LT group were impaired due to deficits in egocentric memory. Further research is needed to examine this possibility and to clarify the use of egocentric and allocentric strategies following thalamic lesions.

Another unresolved issue is the finding that no group, including the Control group, appeared to detect the displacement of objects, or showed a clear preference for novel objects over familiar, at least in terms of direct exploration of the objects. This contrasts with the finding that rats can detect changes in object-place combinations in a very similar task (Save et al., 1992). Several factors, including minor differences in the apparatus used and the strain, sex, prior experience and level of food deprivation of the animals, and the innate preference for some objects over others, may account for this difference. There is a need to examine the influence of these factors on spontaneous object recognition, and to develop and use statistical techniques such as ratio scores that can control for innate object preferences in rats.

A final issue concerns the existence of neural circuits that underpin memory function. Although many studies have investigated the role of thalamic areas in memory, fewer studies have examined the various connections of these structures in neural pathways. Previous research has suggested that the AT, MT and LT may be involved in three separate neural systems that underlie different types of memory. This prediction, however, remains to be tested. Studies using tools such as asymmetrical lesion paradigms and neural tracing would allow these predictions to be tested directly, potentially providing valuable information about the interaction of thalamic areas with other structures. It would also be valuable to have studies which explicitly manipulate or vary the extent of damage and overlap across thalamic regions, including areas (especially midline) adjacent to the AT, MT and LT regions.

4.3 Limitations of the current study

Although one can propose many additional studies or variations that could be employed, it is also important to address the limitations in the behavioural tasks that were conducted. One limitation of the current study is that the testing period for the odour-place paired-associate task was stopped after 14 weeks due to time constraints. During the final weeks of testing, rats in the LT group had

begun to show a small amount of improvement, and it would be interesting to see whether, with continued testing, their performance would improve further. Another issue is that rats were assigned to only one of the simple discrimination tasks, so sample sizes for these tasks were very small. The AT group in the current study seemed to be impaired on the spatial discrimination task, although there were no significant group differences. Larger sample sizes for the simple discrimination tasks would give greater power to detect group differences.

Another limitation is the lack of controls for rats' preferences for various objects in the spontaneous object recognition task. Previous studies have found that rats have an innate preference for some objects over others and have controlled for this (Moran, 2001). Although the current study selected objects that were unable to be climbed into or onto (as rats prefer these types of objects), no pilot study was done to test for object preference. Instead, obviously distinctive objects were used, but the analysis revealed that rats preferred some of these objects over others. Future object recognition studies should take care to control for rats' preference of objects by conducting pilot studies of object preference and/or developing and using ratio scores that can control for object preference.

Another limitation of the current study is that due to time constraints a more detailed analysis of the rats' behaviour on the odour-place paired-associate task and spatial probe tasks could not be completed. A detailed analysis of video data could provide additional information concerning the use of egocentric and allocentric strategies in these tasks.

4.4 General Summary

There are two different views regarding the neural basis of diencephalic amnesia. One view is that a single diencephalic structure is responsible for the memory impairment, whereas the other view is that different structures contribute to the memory deficit in subtly different ways. The current study provided some support for this latter view by demonstrating that AT, MT and LT lesions have different effects on odour-place paired-associate learning. Paired-associate learning tasks are valuable because they provide a way of measuring 'episodic-like' memory in non-human animals. AT and LT lesions severely impaired performance in the current task, whereas MT lesions had no effect. Importantly, the impairment seen after LT lesions was not due to unintentional AT damage. These findings provide further support for the role of the AT in spatial memory, and provide new insight into the role of the MT and LT in paired-associate learning. In addition, the

current study demonstrated that animals may use a combination of egocentric, allocentric and direction cues to solve spatial tasks. This finding highlights the importance of controlling for the use of egocentric strategies on spatial tasks. The role of the AT, MT and LT in spontaneous object recognition was also examined. Although the current study provided no clear picture of the deficits in object recognition after thalamic lesions, it highlights a number of areas for further research on object and object-place recognition.

An important contribution of the current study was that it used highly selective lesions that minimised unintentional damage and overlap between lesions, and used a detailed quantitative analysis to determine the extent of damage caused by these lesions. The use of highly selective lesions and the quantitative analysis of damage are novel, and potentially valuable, tools for future research with thalamic lesions.

With regard to human cases of diencephalic amnesia, the current study, along with previous research, does not support the notion that a single diencephalic area is responsible for amnesia. Instead, it suggests that amnesia may be caused by damage to a number of diencephalic structures, each contributing differently to the overall memory impairment. The current findings suggest that traditional models of memory function (Kesner, 1998; White & McDonald, 2002) may need to be revised to take into account the important role of the thalamic nuclei in memory.

References

Aggleton, J. P., & Brown, M. W. (1999). Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. *Behavioral and Brain Sciences, 22*(3), 425-489.

Aggleton, J. P., Hunt, P. R., Nagle, S., & Neave, N. (1996). The effects of selective lesions within the anterior thalamic nuclei on spatial memory in the rat. *Behavioural Brain Research, 81*(1-2), 189-198.

Aggleton, J. P., Neave, N., Nagle, S., & Hunt, P. R. (1995). A comparison of the effects of anterior thalamic, mamillary body and fornix lesions on reinforced spatial alternation. *Behavioural Brain Research, 68*(1), 91-101.

Aggleton, J. P., & Pearce, J. M. (2001). Neural systems underlying episodic memory: insights from animal research. *Philosophical Transactions of the Royal Society of London: B Biological Sciences, 356*(1413), 1467-1482.

Aggleton, J. P., & Saghal, A. (1993). The contribution of the anterior thalamic nuclei to anterograde amnesia. *Neuropsychologia, 31*(10), 1001-1019.

Alexinsky, T. (2001). Differential effect of thalamic and cortical lesions on memory systems in the rat. *Behavioural Brain Research, 122*(2), 175-191.

Alvarez, P., Wendelken, L., & Eichenbaum, H. (2002). Hippocampal formation lesions impair performance in an odor-odor association task independently of spatial context. *Neurobiology of learning and memory, 78*, 470-476.

Berracochea, D. J., Jaffard, R., & Jarrard, L. E. (1989). Effects of anterior or dorsomedial thalamic ibotenic lesions on learning and memory in rats. *Behavioral and Neural Biology, 51*, 364-376.

Bunsey, M., & Eichenbaum, H. (1993). Critical role of the parahippocampal region for paired-associate learning in rats. *Behavioral Neuroscience, 107*(5), 740-747.

Burk, J. A., & Mair, R. G. (1998). Thalamic amnesia reconsidered: excitotoxic lesions of the intralaminar nuclei, but not the mediodorsal nucleus, disrupt place delayed matching-to-sample performance in rats (Rattus norvegicus). *Behavioral Neuroscience, 112*(1), 54-67.

Byatt, G., & Dalrymple-Alford, J. C. (1996). Both anteromedial and anteroventral thalamic lesions impair radial maze learning in rats. *Behavioral Neuroscience, 110*(6), 1335-1348.

Cheatwood, J. L., Reep, R. L., & Corwin, J. V. (2003). The associative striatum: cortical and thalamic projections to the dorsocentral striatum in rats. *Brain Research, 968*(1), 1-14.

Dalrymple-Alford, J. C. (2005). Animal models of thalamic amnesia: From regional dissociations to recovery of function (pp. Presentation to the Christchurch Brain Research Group, 7 July 2004).

Dix, S. L., & Aggleton, J. P. (1999). Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behavioural Brain Research, 99*, 191-200.

Eichenbaum, H. (1999). The hippocampus and mechanisms of declarative memory. *Behavioural Brain Research, 103*, 123-133.

Eichenbaum, H., Shedlack, K. J., & Eckmann, K. W. (1980). Thalamocortical mechanisms in odor-guided behavior. I. Effects of lesions on the mediodorsal thalamic nucleusand frontal cortex on olfactory discrimination in the rat. *Brain, Behavior and Evolution, 17*(4), 255-275.

Fellows, B. J. (1967). Chance stimulus sequences for discrimination tasks. *Psychological Bulletin, 67*(2), 87-92.

Ferry, A. T., Lu, X. M., & Price, J. L. (2000). Effects of excitotoxic lesions in the ventral striatopallidal–thalamocortical pathway on odor reversal learning: inability to extinguish an incorrect response. *Experimental Brain Research, 131*, 320-335.

Gaffan, D., & Murray, E. A. (1990). Amygdalar interaction with the mediodorsal nucleus of the thalamus and the ventromedial prefrontal cortex in stimulus-reward associative learning in the monkey. *Journal of Neuroscience, 10*(11), 3479-3493.

Gaffan, D., & Parker, A. (2000). Mediodorsal thalamic function in scene memory in rhesus monkeys. *Brain, 123*(4), 816-827.

Gaffan, E. A., Bannerman, D. M., Warburton, E. C., & Aggleton, J. P. (2001). Rats' processing of visual scenes: effects of lesions to fornix, anterior thalamus, mamillary nuclei or the retrohippocampal region. *Behavioural Brain Research, 121*, 103-117.

Gilbert, P. E., & Kesner, R. P. (2002). Role of the rodent hippocampus in paired-associate learning involving associations between a stimulus and a spatial location. *Behavioral Neuroscience, 116*(1), 63-71.

Gilbert, P. E., & Kesner, R. P. (2003). Localization of function within the dorsal hippocampus: the role of the CA3 subregion in paired-associate learning. *Behavioral Neuroscience, 117*(6), 1385-1394.

Groenewegen, H. J. (1988). Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the mediodorsal-prefrontal topography. *Neuroscience, 24*(2), 379-431.

Groenewegen, H. J., & Berendse, H. W. (1994). The specificity of the 'nonspecific' midline and intralaminar thalamic nuclei. *Trends in Neurosciences, 17*(2), 52-57.

Harding, A., Halliday, G., Caine, D., & Kril, J. (2000). Degeneration of anterior thalamic nuclei differentiates alcoholics with amnesia. *Brain, 123*, 141-154.

Harrison, L. M., & Mair, R. G. (1996). A comparison of the effects of frontal cortical and thalamic lesions on measures of spatial learning and memory in the rat. *Behavioural Brain Research, 75*, 195-206.

Hunt, P. R., & Aggleton, J. P. (1998). An examination of the spatial working memory deficit following neurotoxic medial dorsal thalamic lesions in rats. *Behavioural Brain Research, 97*(1-2), 129-141.

Kapur, N., Thompson, S., Cook, P., Lang, D., & Brice, J. (1996). Anterograde but not retrograde memory loss following combined mammillary body and medial thalamic lesions. *Neuropsychologia, 34*(1), 1-8.

Kesner, R. P. (1998). Neurobiological views of memory. In J. L. J. Martinez & R. P. Kesner (Eds.), *Neurobiology of Learning and Memory* (pp. 361-416). San Diego: Academic Press.

Knight, R. G., & Longmore, B. E. (1994). *Clinical Neuropsychology of Alcoholism.* East Sussex: Lawrence Erlbaum Associates Ltd.

Koger, S. M., & Mair, R. G. (1994). Comparison of the effects of frontal cortical and thalamic lesions on measures of olfactory learning and memory in the rat. *Behavioral Neuroscience, 108*(6), 1088-1100.

Kolb, B., Pittman, K., Sutherland, R. J., & Whishaw, I. Q. (1982). Dissociation of the contributions of the prefrontal cortex and dorsomedial thalamic nucleus to spatially guided behavioor in the rat. *Behavioural Brain Research, 6*, 365-378.

Mair, R. G., Burk, J. A., & Porter, M. C. (1998). Lesions of the frontal cortex, hippocampus, and intralaminar thalamic nuclei have distinct effects on remembering in rats. *Behavioral Neuroscience, 112*(4), 772-792.

Mair, R. G., Burk, J. A., & Porter, M. C. (2003). Impairment of radial maze delayed nonmatching after lesions of anterior thalamus and parahippocampal cortex. *Behavioral Neuroscience, 117*(3), 596-605.

Mair, W. G., Warrington, E. K., & Weiskrantz, L. (1979). Memory disorder in Korsakoff's psychosis: a neuropathological and neuropsychological investigation of two cases. *Brain, 102*(4), 749-783.

Mayes, A. R., Meudell, P. R., Mann, D., & Pickering, A. (1988). Location of lesions in Korsakoff's syndrome: neuropsychological and neuropathological data on two patients. *Cortex, 24*(3), 367-388.

McBride, S. A., & Slotnick, B. (1997). The olfactory thalamocortical system and odor reversal learning examined using an asymmetrical lesion paradigm in rats. *Behavioral Neuroscience, 111*(6), 1273-1284.

Mitchell, A. S. (2004). *Involvement of the medial thalamus in multiple attributes of memory.* Unpublished PhD, University of Canterbury, Christchurch.

Mitchell, A. S., & Dalrymple-Alford, J. C. (2004). Multiple memory processing deficits following thalamic injury: Anterior nuclei versus intralaminar/lateral mediodorsal lesions. *FENS Abstracts, 2*(A419.22).

Mitchell, A. S., & Dalrymple-Alford, J. C. (2005). Dissociable memory effects after medial thalamus lesions. *European Journal of Neuroscience (under revision).*

Moran, J. P. (2001). *Comparative effects of perirhinal cortex and anterior thalamic nuclei lesions on radial-maze learning, spontaneous object recognition and configural learning.* Unpublished MSc, University of Canterbury, Christchurch.

Moran, J. P., & Dalrymple-Alford, J. C. (2003). Perirhinal cortex and anterior thalamic lesions: comparative effects on learning and memory. *Behavioral Neuroscience, 007*(6), 1326-1341.

Mumby, D. G., Cameli, L., & Glenn, M. J. (1999). Impaired allocentric spatial working memory and intact retrograde memory after thalamic damage caused by thiamine deficiency in rats. *Behavioral Neuroscience, 113*(1), 42-50.

Newman, L. A., & Burk, J. A. (2004). *Effects of thalamic intralaminar nuclei lesions on attention and working memory in rats.* Paper presented at the Program Number 1002.3 *2004 Abstract viewer/Itinerary planner*, Washington, DC: Society for Neuroscience.

Packard, M. G., & McGaugh, J. L. (1996). Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiology of learning and memory, 65*, 65-72.

Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxic coordinates* (4th ed.). San Diego: Academic Press.

Porter, M. C., Koch, J., & Mair, R. G. (2001). Effects of reversible inactivation of thalamo-striatal circuitry on delayed matching trained with retractable levers. *Behavioural Brain Research, 119*, 61-69.

Savage, L. M., Castillo, R., & Langlais, P. J. (1998). Effects of lesions of thalamic intralaminar and midline nuclei and internal medullary lamina on spatial memory and object discrimination. *Behavioral Neuroscience, 112*(6), 1339-1352.

Savage, L. M., Sweet, A. J., Castillo, R., & Langlais, P. J. (1997). The effects of lesions to thalamic lateral internal medullary lamina and posterior nuclei on learning, memory and habituation in the rat. *Behavioural Brain Research, 82*(2), 133-147.

Save, E., Poucet, B., Foreman, N., & Buhot, M. C. (1992). Object exploration and reactions to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal formation. *Behavioral Neuroscience, 106*(3), 447-456.

Shibata, H. (1998). Organization of projections of rat retrosplenial cortex to the anterior thalamic nuclei. *European Journal of Neuroscience, 10*(10), 3210-3219.

Slotnick, B. M., & Kaneko, N. (1981). Role of mediodorsal thalamic nucleus in olfactory discrimination learning in rats. *Science, 214*, 91-92.

Squire, L. R., & Knowlton, B. J. (2000). The medial temporal lobe, the hippocampus, and the memory systems of the brain. In M. S. Gazzaniga (Ed.), *The New Cognitive Neurosciences* (2 ed.). Cambridge, MA: MIT Press.

Stokes, K. A., & Best, P. J. (1988). Mediodorsal thalamic lesions impair radial maze performance in the rat. *Behavioral Neuroscience, 102*(2), 294-300.

Stokes, K. A., & Best, P. J. (1990a). Mediodorsal thalamic lesions impair "reference" and "working" memory in rats. *Physiology and Behaviour, 47*(3), 471-476.

Stokes, K. A., & Best, P. J. (1990b). Response biases do not underlie the radial maze deficit in rats with mediodorsal thalamus lesions. *Behavioural and Neural Biology, 53*(3), 334-345.

Sutherland, R. J., & Rodriguez, A. J. (1989). The role of the fornix/fimbria and some related subcortical structures in place learning and memory. *Behavioural Brain Research, 32*(3), 265-277.

Sziklas, V., & Petrides, M. (1999). The effects of lesions to the anterior thalamic nuclei on object-place associations in rats. *European Journal of Neuroscience, 11*(2), 559-566.

Van der Werf, Y. D., Jolles, J., Witter, M. P., & Uylings, H. B. (2003). Contributions of thalamic nuclei to declarative memory functioning. *Cortex, 39*(4-5), 1047-1062.

Victor, M., Adams, R. D., & Collins, G. H. (1971). *The Wernicke-Korsakoff Syndrome*. Oxford: Blackwell Scientific Publications.

von Cramon, D. Y., Hebel, N., & Schuri, U. (1985). A contribution to the anatomical basis of thalamic amnesia. *Brain, 108 (Pt 4)*(2), 993-1008.

Warburton, E. C., & Aggleton, J. P. (1999). Differential deficits in the Morris water maze following cytotoxic lesions of the anterior thalamus and fornix transection. *Behavioural Brain Research, 98*(1), 27-38.

Warburton, E. C., Baird, A. L., & Aggleton, J. P. (1997). Assessing the magnitude of the allocentric spatial deficit associated with complete loss of the anterior thalamic nuclei in rats. *Behavioural Brain Research, 87*(2), 223-232.

Warburton, E. C., Morgan, A., Baird, A. L., Muir, J. L., & Aggleton, J. P. (1999). Does pretraining spare the spatial deficit associated with anterior thalamic damage in rats? *Behavioral Neuroscience, 113*(5), 956-967.

Ward-Robinson, J., Wilton, L. A. K., Muir, J. L., Honey, R. C., Vann, S. D., & Aggleton, J. P. (2002). Sensory preconditioning in rats with lesions of the anterior thalamic nuclei: evidence for intact nonspatial 'relational' processing. *Behavioural Brain Research, 133*, 125-133.

White, N. M., & McDonald, R. J. (2002). Multiple parallel memory systems in the brain of the rat. *Neurobiology of learning and memory, 77*(2), 125-184.

Wilton, L. A. K., Baird, A. L., Muir, J. L., Honey, R. C., & Aggleton, J. P. (2001). Loss of the thalamic nuclei for "head direction" impairs performance on spatial memory tasks in rats. *Behavioral Neuroscience, 115*(4), 861-869.

Wood, E. R., Agster, K. M., & Eichenbaum, H. (2004). One-trial odor-reward association: a form of event memory not dependent on hippocampal function. *Behavioral Neuroscience, 118*(3), 526-539.

Young, H. L., Stevens, A. A., Converse, E., & Mair, R. G. (1996). A comparison of temporal decay in place memory tasks in rats (*Rattus Norvegicus*) with lesions affecting thalamus, frontal cortex, or the hippocampal system. *Behavioral Neuroscience, 110*(6), 1244-1260.

Zhang, Y., Burk, J. A., Glode, B. M., & Mair, R. G. (1998). Effects of thalamic and olfactory cortical lesions on continuous olfactory delayed nonmatching-to-sample and olfactory discrimination in rats *(Rattus norvegicus). Behavioral Neuroscience, 112*(1), 39-53.

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15 March 2004

Sheree Gibb
Department of Psychology
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Dear Sheree

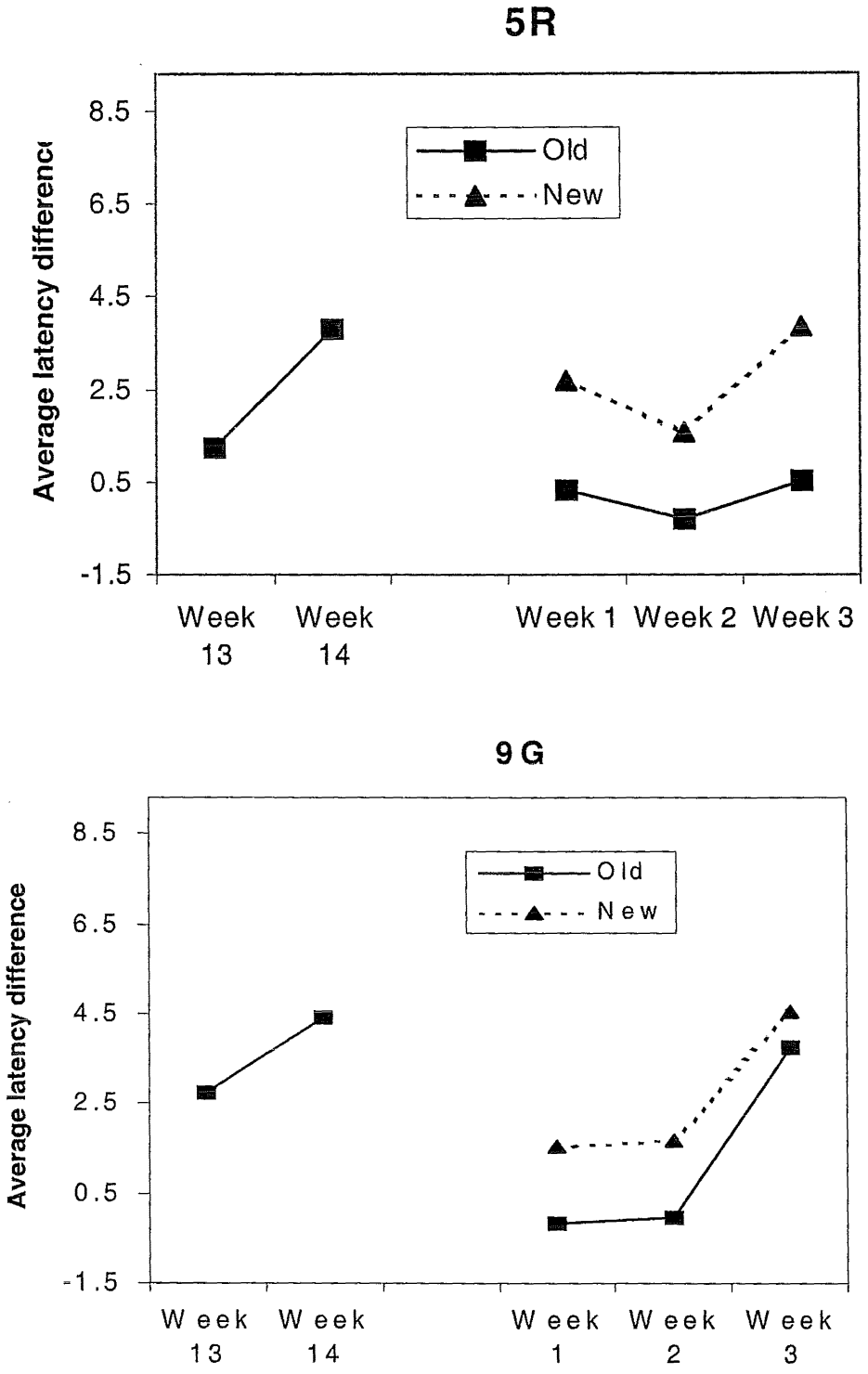
I am pleased to inform you that the Animal Ethics Committee has approved your application entitled: 2004: **5R – "Thalamic nuclei and odour-place association learning."**

Yours sincerely

G. J. Gillon

Dr Gail Gillon
Dean of Science

Appendix A. Ethics approval for the current study.



Appendix B. Individual data for the spatial probe tasks for the two AT rats that showed some evidence of learning on the odour-place paired-associate task.